

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
7 September 2001 (07.09.2001)

PCT

(10) International Publication Number
WO 01/64920 A2

(51) International Patent Classification⁷: **C12N 15/62**,
C07K 14/22, 19/00

Siena (IT). **PIZZA, Mariagrazia** [IT/IT]; Chiron SpA,
Via Fiorentina, 1, I-53100 Siena (IT).

(21) International Application Number: PCT/IB01/00420

(74) Agents: **HALLYBONE, Huw, George et al.**; Carpinaels
& Ransford, 43 Bloomsbury Square, London WC1A 2RA
(GB).

(22) International Filing Date: 28 February 2001 (28.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0004695.3 28 February 2000 (28.02.2000) GB
0027675.8 13 November 2000 (13.11.2000) GB

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,
TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(71) Applicant (*for all designated States except US*): **CHIRON
SPA** [IT/IT]; Via Fiorentina, 1, I-53100 Siena (IT).

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

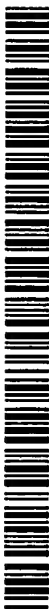
(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **ARICO, Maria**,
Beatrice [IT/IT]; Chiron SpA, Via Fiorentina 1, I-53100
Siena (IT). **COMANDUCCI, Maurizio** [IT/IT]; Chiron
SpA, Via Fiorentina 1, I-53100 Siena (IT). **GALEOTTI**,
Cesira [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100
Siena (IT). **MASIGNANI, Vega** [IT/IT]; Chiron SpA, Via
Fiorentina, 1, I-53100 Siena (IT). **GIULIANI, Marzia**,
Monica [IT/IT]; Chiron SpA, Via Fiorentina, 1, I-53100

Published:

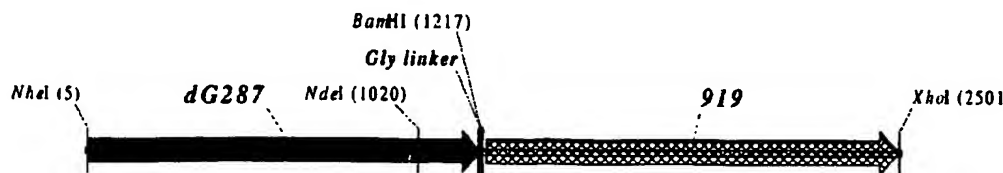
— without international search report and to be republished
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/64920 A2

(54) Title: HYBRID EXPRESSION OF NEISSERIAL PROTEINS



(57) Abstract: Two or more Neisserial proteins (e.g. A and B) are expressed as a single hybrid protein which can be represented simply by the formula NH₂-A-B-COOH.

HYBRID EXPRESSION OF NEISSERIAL PROTEINS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of protein expression. In particular, it relates to the heterologous
5 expression of proteins from *Neisseria* (e.g. *N.gonorrhoeae* or, preferably, *N.meningitidis*).

BACKGROUND ART

International patent applications WO99/24578, WO99/36544, WO99/57280 and
WO00/22430 disclose proteins from *Neisseria meningitidis* and *Neisseria gonorrhoeae*.
These proteins are typically described as being expressed in *E.coli* (i.e. heterologous
10 expression) as either N-terminal GST-fusions or C-terminal His-tag fusions, although other
expression systems, including expression in native *Neisseria*, are also disclosed.

It is an object of the present invention to provide alternative and improved approaches for
the heterologous expression of these proteins. These approaches will typically affect the
level of expression, the ease of purification, the cellular localisation of expression, and/or the
15 immunological properties of the expressed protein.

DISCLOSURE OF THE INVENTION

In accordance with the invention, two or more (e.g. 3, 4, 5, 6 or more) proteins of the
invention are expressed as a single hybrid protein. It is preferred that no non-Neisserial
fusion partner (e.g. GST or poly-His) is used.

20 This offers two advantages. Firstly, a protein that may be unstable or poorly expressed on its
own can be assisted by adding a suitable hybrid partner that overcomes the problem.
Secondly, commercial manufacture is simplified – only one expression and purification need
be employed in order to produce two separately-useful proteins.

Thus the invention provides a method for the simultaneous heterologous expression of two
25 or more proteins of the invention, in which said two or more proteins of the invention are
fused (i.e. they are translated as a single polypeptide chain).

The method will typically involve the steps of: obtaining a first nucleic acid encoding a first
protein of the invention; obtaining a second nucleic acid encoding a second protein of the

invention; ligating the first and second nucleic acids. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector.

Where just two proteins are joined, the hybrid protein can be represented simply by the formula $\text{NH}_2\text{-A-B-COOH}$. A and B can each be selected from any Neisserial proteins, and in particular those represented by SEQ#s 1-4326. The method is well suited to the expression of proteins orf1, orf4, orf25, orf40, Orf46/46.1, orf83, 233, 287, 292L, 564, 687, 741, 907, 919, 953, 961 and 983.

The 42 hybrids indicated by 'X' in the following table of form $\text{NH}_2\text{-A-B-COOH}$ are preferred:

A → B	ORF46.1	287	741	919	953	961	983
ORF46.1		X	X	X	X	X	X
287	X		X	X	X	X	X
741	X	X		X	X	X	X
919	X	X	X		X	X	X
953	X	X	X	X		X	X
961	X	X	X	X	X		X
983	X	X	X	X	X	X	

Preferred proteins to be expressed as hybrids are thus ORF46.1, 287, 741, 919, 953, 961 and 983. These may be used in their essentially full-length form, or poly-glycine deletions (ΔG) forms may be used (e.g. $\Delta\text{G-287}$, ΔGTbp2 , ΔG741 , ΔG983 etc.), or truncated forms may be used (e.g. $\Delta\text{1-287}$, $\Delta\text{2-287}$ etc.), or domain-deleted versions may be used (e.g. 287B, 287C, 287BC, ORF46₁₋₄₃₃, ORF46₄₃₃₋₆₀₈, ORF46, 961c etc.) and so on.

Particularly preferred are: (a) a hybrid protein comprising 919 and 287; (b) a hybrid protein comprising 953 and 287; (c) a hybrid protein comprising 287 and ORF46.1; (d) a hybrid protein comprising ORF1 and ORF46.1; (e) a hybrid protein comprising 919 and ORF46.1; (f) a hybrid protein comprising ORF46.1 and 919; (g) a hybrid protein comprising ORF46.1, 287 and 919; (h) a hybrid protein comprising 919 and 519; and (i) a hybrid protein comprising ORF97 and 225.

Further embodiments are shown in the drawings and include $\Delta\text{G287-919}$, $\Delta\text{G287-953}$, $\Delta\text{G287-961}$, $\Delta\text{G983-ORF46.1}$, $\Delta\text{G983-741}$, $\Delta\text{G983-961}$, $\Delta\text{G983-961C}$, $\Delta\text{G741-961}$, $\Delta\text{G741-961C}$, $\Delta\text{G741-983}$, $\Delta\text{G741-ORF46.1}$, ORF46.1-741, ORF46.1-961, ORF46.1-961C,

961-ORF46.1, 961-741, 961-983, 961C-ORF46.1, 961C-741, 961C-983, 961CL-ORF46.1, 961CL-741, and 961CL-983.

Where 287 is used, it is preferably at the C-terminal end of a hybrid; if it is to be used at the N-terminus, it is preferred to use a ΔG form of 287 is used (*e.g.* as the N-terminus of a hybrid with ORF46.1, 919, 953 or 961).

Where 287 is used, this is preferably from strain 2996 or from strain 394/98.

Where 961 is used, this is preferably at the N-terminus. Domain forms of 961 may be used.

Alignments of polymorphic forms of ORF46, 287, 919 and 953 are disclosed in WO00/66741. Any of these polymorphs can be used according to the present invention.

10 Preferably, the constituent proteins (A and B) in a hybrid protein according to the invention will be from the same strain.

The fused proteins in the hybrid may be joined directly, or may be joined via a linker peptide *e.g.* via a poly-glycine linker (*i.e.* G_n where $n = 3, 4, 5, 6, 7, 8, 9, 10$ or more) or via a short peptide sequence which facilitates cloning. It is evidently preferred not to join a ΔG protein to the C-terminus of a poly-glycine linker.

The fused proteins may lack native leader peptides or may include the leader peptide sequence of the N-terminal fusion partner.

Host

It is preferred to utilise a heterologous host. The heterologous host may be prokaryotic or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (*e.g.* *M.tuberculosis*), yeast *etc.*

Vectors, hosts etc.

25 As well as the methods described above, the invention provides (a) nucleic acid and vectors useful in these methods (b) host cells containing said vectors (c) proteins expressed or expressable by the methods (d) compositions comprising these proteins, which may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions (e) these compositions for use as medicaments (*e.g.* as vaccines) or as diagnostic reagents (f)

the use of these compositions in the manufacture of (1) a medicament for treating or preventing infection due to Neisserial bacteria (2) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria, and/or (3) a reagent which can raise antibodies against Neisserial bacteria and (g) a method of treating a patient, comprising administering to the patient a therapeutically effective amount of these compositions.

Sequences

The invention also provides a protein or a nucleic acid having any of the sequences set out in the following examples. It also provides proteins and nucleic acid having sequence identity to these. As described above, the degree of 'sequence identity' is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more).

Nomenclature herein

The 2166 protein sequences disclosed in WO99/24578, WO99/36544 and WO99/57280 are referred to herein by the following SEQ# numbers:

Application	Protein sequences	SEQ# herein
WO99/24578	Even SEQ IDs 2-892	SEQ#s 1-446
WO99/36544	Even SEQ IDs 2-90	SEQ#s 447-491
WO99/57280	Even SEQ IDs 2-3020	SEQ#s 492-2001
	Even SEQ IDs 3040-3114	SEQ#s 2002-2039
	SEQ IDs 3115-3241	SEQ#s 2040-2166

In addition to this SEQ# numbering, the naming conventions used in WO99/24578, WO99/36544 and WO99/57280 are also used (*e.g.* 'ORF4', 'ORF40', 'ORF40-1' *etc.* as used in WO99/24578 and WO99/36544; 'm919', 'g919' and 'a919' *etc.* as used in WO99/57280).

The 2160 proteins NMB0001 to NMB2160 from Tettelin *et al.* [*Science* (2000) 287:1809-1815] are referred to herein as SEQ#s 2167-4326 [see also WO00/66791].

The term 'protein of the invention' as used herein refers to a protein comprising:

- (a) one of sequences SEQ#s 1-4326; or
- (b) a sequence having sequence identity to one of SEQ#s 1-4326; or
- (c) a fragment of one of SEQ#s 1-4326.

The degree of 'sequence identity' referred to in (b) is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more). This includes mutants and allelic variants [e.g. see WO00/66741]. Identity is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence.

The 'fragment' referred to in (c) should comprise at least *n* consecutive amino acids from one of SEQ#s 1-4326 and, depending on the particular sequence, *n* is 7 or more (eg. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). Preferably the fragment comprises an epitope from one of SEQ#s 1-4326. Preferred fragments are those disclosed in WO00/71574 and WO01/04316.

Preferred proteins of the invention are found in *N.meningitidis* serogroup B.

Preferred proteins for use according to the invention are those of serogroup B *N.meningitidis* strain 2996 or strain 394/98 (a New Zealand strain). Unless otherwise stated, proteins mentioned herein are from *N.meningitidis* strain 2996. It will be appreciated, however, that the invention is not in general limited by strain. References to a particular protein (e.g. '287', '919' etc.) may be taken to include that protein from any strain.

It will be appreciated that references to "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) etc.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1 to 26 show hybrid proteins according to the invention.

MODES FOR CARRYING OUT THE INVENTION

25 *Example 1 – hybrids of ORF46*

The complete ORF46 protein from *N.meningitidis* (serogroup B, strain 2996) has the following sequence:

30 1 LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRO HFEPDGKYHL
 51 FGSRGELAER SGHIGLGKIQ SHQLGNLMIQ QAAIKGNIGY IVRFSDHGHE
 101 VHSPFDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHHPAD GYDGPQGGGY

151 PAPKGARDIY SYDIKGVAQN IRLNLTNRS TGQRLADRFH NAGSMLTQGV
 201 GDGFKRATRY SPELDRSGNA AEAFTGTADI VKNIIGAAGE IVGAGDAVQG
 251 ISEGSNIAVM HGLGLLSTEN KMARINDLAD MAQLKDYAAA AIRDWAVQNP
 301 NAAQGIEAVS NIFMAAIPK GIGAVRGKYG LGGITAHPIK RSQMGAIALP
 351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI TSSTVPPSNG
 401 KNVKLADQRH PKTGVPPFDGK GFPNFEKHVK YDTKLDIQEL SGGGIPKAKP
 451 VSDAKPRWEV DRKLNKLTTR EQVEKNVQEI RINGNKNSNFS QHAQLEREIN
 501 KLKSADEINF ADGMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
 551 KAYIVRGNNR VFAAEYLGRI HELKFKKVDF FVPNTSWKNP TDVLNESGNV
 601 KRPRYRSK*

The leader peptide is underlined.

The sequences of ORF46 from other strains can be found in WO00/66741.

ORF46 has been fused at its C-terminus and N-terminus with 287, 919, and ORF1. The hybrid proteins were generally insoluble, but gave some good ELISA and bactericidal results (against the homologous 2996 strain):

Protein	ELISA	Bactericidal Ab
Orf1-Orf46.1-His	850	256
919-Orf46.1-His	12900	512
919-287-Orf46-His	n.d.	n.d.
Orf46.1-287His	150	8192
Orf46.1-919His	2800	2048
Orf46.1-287-919His	3200	16384

For comparison, 'triple' hybrids of ORF46.1, 287 (either as a GST fusion, or in Δ G287 form) and 919 were constructed and tested against various strains (including the homologous 2996 strain) *versus* a simple mixture of the three antigens. FCA was used as adjuvant:

	2996	BZ232	MC58	NGH38	F6124	BZ133
Mixture	8192	256	512	1024	>2048	>2048
ORF46.1-287-919his	16384	256	4096	8192	8192	8192
Δ G287-919-ORF46.1his	8192	64	4096	8192	8192	16384
Δ G287-ORF46.1-919his	4096	128	256	8192	512	1024

Again, the hybrids show equivalent or superior immunological activity.

Hybrids of two proteins (strain 2996) were compared to the individual proteins against various heterologous strains:

	1000	MC58	F6124 (MenA)
ORF46.1-His	<4	4096	<4
ORF1-His	8	256	128
ORF1—ORF46.1-His	1024	512	1024

Again, the hybrid shows equivalent or superior immunological activity.

Example 2 – hybrids of ΔG287

The deletion of the (Gly)₆ sequence in 287 was found to have a dramatic effect on protein expression. The protein lacking the N-terminal amino acids up to GGGGGG is called 'ΔG287'. In strain MC58, its basic sequence (leader peptide underlined) is:

5
 10
 15
 20
 25
 30
 35
 40

```

SPDVKS ADTLSKPAAP VVSEKETEAQ EDAPQAGSQ QGAPSAQGSQ DMAAVSEENT
GNGGAVTADN PKNEDEVAQN DMPQNAAGTD SSTPNHTPDP NMLAGNMENQ ATDAGESSQP
ANQPDMANAA DGMQGGDDPSA GGQNAGNTAA QGANQAGNNQ AAGSSDPIPA SNPAPANGGS
NFGRVDLANG VLIDGPSQNI TLTHCKGDSC SGNNFLDEEV QLKSEFEKLS DADKISNYKK
DGKNDKFGVL VADSVQMKGI NQYIIFYKPK PTSFARFRRS ARSRRLPAE MPLIPVNQAD
TLIVDGEAVS LTGHSGNIFA PEGNYRYLTY GAEKLPGGSY ALRVQGEPAK GEMLAGAAVY
NGEVLHFPTE NGRPYPTRGR FAKVDFGSK SVDGIIDSGD DLHMGTKQFK AAIDGNGFKG
TWTENGSGDV SGKFGYPAGE EVAGKYSYRP TDAEKGFGV FAGKKEQD*
  
```

ΔG287, with or without His-tag ('ΔG287-His' and 'ΔG287K', respectively), are expressed at very good levels in comparison with the '287-His' or '287^{untagged}'.

On the basis of gene variability data, variants of ΔG287-His were expressed in *E.coli* from a number of MenB strains, in particular from strains 2996, MC58, 1000, and BZ232. The results were also good – each of these gave high ELISA titres and also serum bactericidal titres of >8192. ΔG287K, expressed from pET-24b, gave excellent titres in ELISA and the serum bactericidal assay.

Deletion of poly-Gly sequences is also applicable to Tbp2 (NMB0460), 741 (NMB 1870) and 983 (NMB1969). When cloned in pET vector and expressed in *E.coli* without the sequence coding for their leader peptides and without poly-Gly (*i.e.* as "ΔG forms"), the same effect was seen – expression was good in the clones carrying the deletion of the poly-glycine stretch, and poor or absent if the glycines were present in the expressed protein.

ΔG287 was fused directly in-frame upstream of 919, 953, 961 (sequences shown below) and ORF46.1:

30
 35
 40

```

ΔG287-919
ATGGCTAGCCCCGATGTTAAATCGGCGGACACGCTGTCAAACCGGCCGCTCCTGTTGTTGCTGAAAAAGAGACAGAG
GTAAAAGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCACACAAGGCAGCCAAGATATGGCGGCA
GTTTCGGCAGAAAATACAGGCAATGGCGGTGCGGCAACAACGGACAAACCCAAAAATGAAGACGAGGGACCGCAAAAT
GATATGCCGCAGAAAATTCGCCGAATCCGCAAAATCAAACAGGGAACAACCAACCGCCGATTCTTCAGATTCCGCCCCC
GCGTCAAACCTGCACCTGCGAATGGCGGTAGCAATTTTGGAAGGGTTGATTGGCTAATGGCGTTTGTATTGATGGG
CCGTCGCAAAATATAACGTGACCCACTGTAAAGGCGATTCTTGTAATGGTGATAATTTATTGGATGAAGAAGCACCG
TCAAATCAGAATTTGAAAATTTAAATGAGTCTGAACGAATTGAGAAATATAAGAAAGATGGGAAAAGCGATAAAATTT
ACTAATTTGGTTGCGACAGCAGTTCAAGCTAATGGAACATAACAAATATGTATCATCATTTATAAAGACAAGTCCGCTTCA
TCTTCATCTGCGCGATTAGGCGTTCTGCACGGTCGAGGAGGTCGCTTCCTGCCGAGATGCCGCTAATCCCGCTCAAT
CAGGCGGATACGCTGATTGTGATGGGGAAGCGGTCAGCCTGACGGGGCATTCCGGCAATATCTTCGCGCCCCGAAGGG
AATTACCGGTATCTGACTTACGGGGCGGAAAAATTGCCCGCGGATCGTATGCCCTCCGTGTGCAAGGCGAACCGGCA
AAAGGCGAAATGCTTGCTGGCACGGCCGTGTACAACGGCGAAGTGCTGCATTTTCATACGGAACCGCCGTCCGTAC
CCGACTAGAGGCAGGTTTGCCGCAAAAGTCGATTTCGGCAGCAAAATCTGTGGACGGCATTATCGACAGCGCGGATGAT
TTCATATGGGTACGCAAAATTCAAAGCCGCCATCGATGGAACGGCTTTAAGGGGACTTGGACGGAATAATGGCGGC
GGGATGTTTCGGAAGGTTTACGGCCCGCGCCGCGAGGAAGTGCGGGGAAAATACAGCTATCGCCCGACAGATGCG
  
```


5 GAAAAGGGCGGATTCGGCGTGTTCGCGGCAAAAAAGAGCAGGATGGATCCGGAGGAGGAGGATGCCAAAGCAAGAGC
ATCCAAACCTTTCCGCAACCCGACACATCCGTCATCAACGGCCCGGACCGCGCGTTCGGCATCCCGACCCCGCGCGGA
ACGACGGTTCGGCGCGCGCGGGCGCTCTATACCGTGTACCGCACCTGTCCCTGCCCACTGGGGCGCGCAGGATTTTC
GCCAAAAGCCTGCAATCCTTCCGCTCGGCTGCGCCAATTTGAAAAACCGCAAGGCTGGCAGGATGTGTGCGCCCAA
GCCTTTCAAACCCCGTCCATTCTTTTCAGGCAAAACAGTTTGTGAACGCTATTTACGCGCGTGGCAGGTTGACAGGC
AACGGAAGCCTTGGCGGTACGGTTACCGGCTATTACGAGCCGGTGTGAAGGGCGACGACAGGCGGACGGCACAAGCC
CGCTTCCCGATTTACGGTATTTCCGACGATTTTATCTCCGTCCCCCTGCTGCGGTTTGGCGGAGCGGAAAAGCCCTT
GTCCGCATCAGGCAGACGGGAAAAACAGCGGCACAATCGACAATACCGGCGGCACACATACCGCCGACCTCTCCCGA
TTCCCCATCACCGCGCGCACAAACGGCAATCAAAGGCAGGTTTGAAGGAAGCCGCTTCTCCCCCTACCACACGCGCAAC
CAAATCAACGGCGCGCGCTTGACGGCAAAGCCCCGATACTCGGTTACGCCGAAGACCCCGTTCGAACCTTTTTTTTATG
CACATCCAAGGCTCGGGCGCTGTGAAAACCCCGTCCGGCAAATACATCCGCATCGGCTATGCCGACAAAAACGAACAT
CCCTACGTTTCCATCGGACGCTATATGGCGGACAAAGGCTTACCTCAAGCTCGGGCAGACCTCGATGCAGGCGATCAAA
GCCTATATGCGGCAAAATCCGCAACGCCTCGCCGAAGTTTGGGTCAAACCCCGATATATCTTTTCCGCGAGCTT
15 GCGGAAGCAGCAATGACGGTCCCGTTCGGCGCACTGGGCACGCCGTTGATGGGGGAATATGCCGGCGCAGTCGACCGG
CACTACATTACCTTGGGCGCGCCCTTATTTGTCCGACCGCCCATCCGGTTACCCGCAAAGCCCTCAACCGCCTGATT
ATGGCGCAGGATACCGGCAGCGGATTAAGGCGCGGTGCGCGTGGATTATTTTGGGGATACGGCGACGAAGCCGGC
GAACTTGCCGGCAACAGAAAACACGGGTACGCTTGGCAGCTCCTACCCAACGGTATGAAGCCCGAATACCGCCCG
TAACTCGAG

20 1 MASPDVKSAD TLSKPAAPVV AEKETE VKED APQAGSQGG APSTQGSQDM
51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNQPADSS
101 DSAPASNAP ANGGSNFRV DLANGVLIDG PSQNTITLTHC KGDSCNGDNL
151 LDEEAPSKSE FENLNESERI EKYKDKGSD KFTNLVATAV QANGTNKYVI
201 IYKDKSASS SARFRSARS RRLPAEMPL IPVNQADTLI VDGEAVSLTG
25 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKGEM LAGTAVYNGE
301 VLHFHTENGR PYPTRGRFAA KVDGFSKSD GIIDSGDDLH MGTQKPKAAI
351 DGNFGKTWT ENGCGDVSGR FYGPAGEEVA GKYSYRPTDA EKGFGVVFAG
401 KKEQDGS GGG GCQSKSIQTF PQPDTSVING PDRFVGIPDP AGTTVCGGGGA
451 VYTVPHLSL PHWAAQDFAK SLQSFRLGCA NLKNRQGWQD VCAQAPQTFV
30 501 HSFQAKOFFE RYFTPWQVAG NGSLAGTVTG YYEPVLKGDD RRTAQARFPI
551 YGIPDDFISV PLPAGLRSGK ALVRIROTGK NSGTIDNTGG THTADLSRFP
601 ITARTTAIKG RFEGSRFLPY HTRNQINGGA LDGKAPILGY AEDPVELFFM
651 HIQSGRLKT PSGYIRIGY ADKNEHPYVS IGRYMADKGY LKLGQTSMQG
701 IKAYMRQNPQ RLAEVLQNP SYIFFRELAG SSNDGPVGAL GTPLMGEYAG
35 751 AVDRHYITLG APLFVATAHP VTRKALNRLI MAQDTGSAIK GAVRVDYFWG
801 YGDEAGELAG KQKTTGYVWQ LLPNGMKPEY RP*

40 AG287-953
ATGGCTAGCCCCGATGTTAAATCGGCGGACACGCTGTCAAACCGGCCGCTCCTGTTGTTGCTGAAAAAGAGACAGAG
GTAAAAGAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGCGCCATCCACACAAGGCAGCCAAGATATGGCGGCA
GTTTCGGCAGAAAATACAGGCAATGGCGGTGCGGCAACACGGAACCAACCAAAATGAAGACGAGGACCGCAAAAT
GATATGCCGCAAAATTCGCGCAATCCGCAATCAAACAGGGAACAACCAACCGCCGATTCTTCAGATTCCGCCCCC
45 GCGTCAAACCCCTGCACCTGCGAATGGCGGTAGCAATTTTGAAGGGTTGATTGTTGCTAATGGCGTTTGTATTGATGGG
CCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGTAAATGGTGATAATTTATTGGATGAAGAAGCACCG
TCAAATCAGAATTTGAAAATTTAAATGAGTCTGAACGAATTGAGAAATATAAGAAAGATGGGAAAAGCGATAAATTT
ACTAATTTGGTTGCGACAGCAGTTCAAGCTAATGGAACATAAATATGTCATCATTTATAAAGACAAGTCCGCTTCA
TCTTCATCTGCGGATTTCAGGCGTTCTGACGGTTCGAGGAGGTGCGTTCTCTGCGGAGATGCCGCTAATCCCCGTCAAT
50 CAGGCGGATACGCTGATTGTGATGGGGAAGCGGTACGCTGACGGGCATTTCGGCAATATCTTCGCGCCCCGAAGGG
AATTACCGGTATCTGACTTACGGGGCGGAAAAATTGCCCGCGGATCGTATGCCCTCCGTGTGCAAGGCGAACCGGCA
AAAGGCGAAATGCTTGTGTCACGGCCGTGTACAACGGCGAAGTGTGCTGATTTTCATACGGAAAACGGCCGTCCGTAC
CCGACTAGAGGCAGGTTTGGCGCAAAAGTCGATTTCGGCAGCAAAATCTGTGGACGGCATTATCGACAGCGGCGATGAT
TTGCATATGGGTACGCAAAATTCAAAGCCGCATCGATGAAACCGGCTTAAAGGGACTTGGACGGAATGGCGGC
55 GGGGATGTTTCCGGAAGGTTTACGGCCCGCGCGGAGGAGTGGCGGGAATAACGCTATCGCCCGACAGATGCG
GAAAAGGGCGGATTTCGGCGTGTTCGCGGCAAAAAAGAGCAGGATGGATCCGGAGGAGGAGGAGCCACTACAAAGTG
GACGAATATCACGCCAACGCCGTTTCGCCATCGACCATTTCAACACCAGCACCAACGTCGGCGGTTTTTACGGTCTG
ACCGGTTCCGTCGAGTTCGACCAAGCAAAACGCGACGGTAAAATCGACATCACCATCCCCGTGCAACCTGCAAGC
GGTTCGCAACACTTTACCGACCACCTGAAATCAGCCGACATCTTCGATGCCGCCCAATATCCGGACATCCGCTTTGTT
60 TCCACCAAAATCAACTTCAACGGCAAAAACTGGTTTCCGTTGACGGCAACCTGACCATGCACGGCAAAACCGCCCCC
GTCAAACCTCAAAGCCGAAAAATCAACTGCTACCAAGCCGATGCGGGAACCGAAGTTTGGCGGCGGACATTCAGC
ACCACCATCGACCGACCAATGGGGCGTGACTACCTCGTTAAGTTGGTATGACCAAAAGCGTCCGCATCGACATC
CAAATCGAGGCAGCCAAACAATAACTCGAG

65 1 MASPDVKSAD TLSKPAAPVV AEKETE VKED APQAGSQGG APSTQGSQDM
51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNQPADSS
101 DSAPASNAP ANGGSNFRV DLANGVLIDG PSQNTITLTHC KGDSCNGDNL
151 LDEEAPSKSE FENLNESERI EKYKDKGSD KFTNLVATAV QANGTNKYVI

-9-

5

201 IYKDKSASSS SARFRRSARS RRSLPAEMPL IPVNQADTLI VDGEAVSLTG
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKGEM LAGTAVYNGE
 301 VLHFHTENGR PYPTRGRFAA KVDGFSKSD GIIDSGDDLH MGTQKPKAAI
 351 DGNFGKGTWT ENGGGDVSGR FYGPAGEEVA GKYSYRPTDA EKGFGVFAG
 401 KKEQDGS GGGG GATYKVDEYH ANARFAIDHF NTSTNVGGFY GLTGSVEFDQ
 451 AKRDGKIDIT IPVANLQSGS QHFTDHLKSA DIFDAAQYPD IRFVSTKFNF
 501 NGKKLVSDG NLTMHGKTAP VKLKAKEFNC YQSPMAKTEV CGGDFSTTID
 551 RTRKWGVLDLV NVGMTKSVRI DIQIEAAQ*

10

ΔG287-961

15

20

25

30

35

40

ATGGCTAGCCCCGATGTTAAATCGGCGGACACGCTGTCAAACCGGCCGCTCCTGTTGTTGCTGAAAAAGAGACAGAG
 GTAAAGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCACACAAGGCAGCCAAGATATGGCGGCA
 GTTTTCGGCAGAAAATACAGGCAATGGCGGTGCGGCAACAACGGACAAACCCAAAAATGAAGACGAGGGACCGCAAAAT
 GATATGCCGCAAAATTCGCGCGAATCCGCAATCAACAGGGAACAACCAACCGCCGATTCTTCAGATTCCGCCCCC
 GCGTCAAACCTGCACCTGCGAATGGCGGTAGCAATTTTGGGAAGGGTTGATTGGCTAATGGCGTTTTGATTGATGGG
 CCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGGATTCTTGTAATGGTGATAATTTATTGGATGAAGAAGCACC
 TCAAAATCAGAATTTGAAAATTTAAATGAGTCTGAACGAATTGAGAAATATAAGAAAGATGGGAAAAGCGATAAATTT
 ACTAATTTGGTTGCGACAGCAGTTCAAGCTAATGGAACTAACAATATGTATCATATTATAAAGACAAGTCCCGCTTCA
 TCTTCATCTGCGCGATTTCAGGCGTTCTGACGCGTTCGAGGAGTTCGCTTCCTGCGGAGATGCGCTAATCCCGCTCAAT
 CAGGCGGATACGCTGATTGTGATGGGGAAGCGGTGACGCTGACGGGGCATTCCGGCAATATCTTCGCGCCCGAAGGG
 AATTACCGGTATCTGACTTACGGGGCGGAAAAATTGCCGCGCGGATCGTATGCCCTCCGTTGTGCAAGGCGAACCGGCA
 AAAGGCGAAATGCTTGCTGGCAGCGCCGTGTACAACGGCGAAGTGCTGCATTTTCATACGGAACCGCCGTCGCTAC
 CCGACTAGAGGCGAGTTTGCCGCAAAAGTCGATTTCCGCGAGCAAAATCTGTGGACGGCATTATCGACAGCGCGGATGAT
 TTGCATATGGGTACGCAAAAATTCAAAGCCGCCATCGATGGAAACGGCTTTAAGGGGACTTGGACGGAATGGCGGC
 GGGGATGTTTTCCGGAAGGTTTTACGGCCCGCGCGGAGGAGTGGCGGAAAAATACAGCTATCGCCCGACAGATGCG
 GAAAAGGGCGGATTTCGCGGTGTTTGCCGGCAAAAAGAGCAGGATGGATCCGGAGGAGGAGGACCAACGACGAC
 GATGTTAAAAAGCTGCCACTGTGCCATTGCTGCTGCCATACAACAATGGCCAAGAAATCAACGGTTTTCAAAGCTGGA
 GAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGAC
 TTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAAACAAACAAACGTCGATGCC
 AAGTAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATACT
 GATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAAGAGACTAAG
 ACAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTCAACGAT
 ATCGCCGATTCTATTGGATGAAACCAACACTAAGGCAGACGAGCCGTCAAACCGCCAATGAAGCCAAACAGACGGCC
 GAAGAAACCAACAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAGCCGAAGCTCCGCTGGC
 ACAGCTAATACTGCAGCCGACAAGCCGAGCTGCTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAAC
 AAAGATAATATTGCTAAAAAAGCAACAGTGCAGCAGTGTACACCAGAGAAGAGTCTGACAGCAAAATTTGTGAGAATT
 GATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATCAGGATACT
 CGCCTGAACGGTTTTGGATAAAACAGTGTGACACTGCGCAAGAAACCCGCCAAGGCTTGCAGAAACAGCCGCGCTC
 TCCGGTCTGTTCCAACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCCGGCGGTACAAATCCGAATCGGCA
 GTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACTTTGCCGCAAAAGCAGGCGTGGCAGTCCGCACTTCGTCGGGT
 TCTTCCGACGCTACCATGTGCGCGTCAATTACGAGTGGTAACTCGAG

45

50

55

60

1 MASPDVKSAD TLSKPAAPVV AEKETEVED APQAGSQGQ APSTQGSQDM
 51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNQPADSS
 101 DSAPASNAP ANGGSNFRV DLANGVLIDG PSQNTLTHC KGDSCNGDNL
 151 LDEEAPSKSE FENLNESERI EKYKDKGSD KFTNLVATAV QANGTNKYVI
 201 IYKDKSASSS SARFRRSARS RRSLPAEMPL IPVNQADTLI VDGEAVSLTG
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKGEM LAGTAVYNGE
 301 VLHFHTENGR PYPTRGRFAA KVDGFSKSD GIIDSGDDLH MGTQKPKAAI
 351 DGNFGKGTWT ENGGGDVSGR FYGPAGEEVA GKYSYRPTDA EKGFGVFAG
 401 KKEQDGS GGGG GATNDDVVK AATVAIAAAY NNGQEINGFK AGETIYDIDE
 451 DGTITKKDAT AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDAKVKAEE
 501 SEIEKLTTKL ADTDAALADT DAALDATNA LNKLGENTIT FAEETKTNIV
 551 KIDEKLEAVA DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE
 601 TKQNVDAKV AAETAAGKAE AAAGTANTAA DKABAVAARKV TDIKADIATN
 651 KDNIAKKANS ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH
 701 DTRLNGLDKT VSDLRKETRQ GLAEQAALSG LFQPYNVGRF NVTAAVGGYK
 751 SESAVAIGTG FRFTENFAAK AGVAVGTSSG SSAAYHVGVN YEW*

	ELISA	Bactericidal
Δ G287-953-His	3834	65536
Δ G287-961-His	108627	65536

The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens (using 287-GST) for 919 and ORF46.1:

	Mixture with 287	Hybrid with Δ G287
919	32000	128000
ORF46.1	128	16000

Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained:

	919		ORF46.1	
Strain	Mixture	Hybrid	Mixture	Hybrid
NGH38	1024	32000	-	16384
MC58	512	8192	-	512
BZ232	512	512	-	-
MenA (F6124)	512	32000	-	8192
MenC (C11)	>2048	>2048	-	-
MenC (BZ133)	>4096	64000	-	8192

The hybrid proteins with Δ G287 at the N-terminus are therefore immunologically superior to simple mixtures, with Δ G287-ORF46.1 being particularly effective, even against heterologous strains. Δ G287-ORF46.1K may be expressed in pET-24b.

The same hybrid proteins were made using New Zealand strain 394/98 rather than 2996:

10

 Δ G287NZ-919

ATGGCTAGCCCCGATGTCAAGTCGGCGGACACGCTGTCAAAACCTGCCGCCCTGTTGTTTCTGAAAAAGAGACAGAG
 GCAAAGGAAGATGCCCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCGCACAAGGCGGTCAAGATATGGCGGCG
 GTTTCGGAAGAAATACAGGCAATGGCGGTGCGGCAGCAACGACAAACCCAAAAATGAAGACGAGGGGGCGCAAAAT
 GATATGCCGCAAAATGCCGCCGATACAGATAGTTTGACACCGAATCACACCCCGGCTTCGAATATGCCGCCCGGAAAT
 ATGGAACCAAGCACCCGATGCCGGGAAATCGGAGCAGCCGGCAAACCAACCGGATATGGCAAATACGGCGGACGGA
 ATGCAGGGTGACGATCCGTCGGCAGGCGGGGAAATGCCGGCAATACGGCTGCCCAAGGTACAAATCAAGCCGAAAC
 AATCAAAACCGCGGTTCTCAAAATCCTGCCTCTTCAACCAATCCTAGCGCCACGAATAGCGGTGGTGATTTTGGAAGG
 ACGAACGTGGGCAATCTGTTGTGATTGACGGGCCGTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGT
 AGTGGCAATAATTTCTTGGATGAAGAAGTACAGCTAAATCAGAATTTGAAAAATTAAAGTGATGCAGACAAATAAGT
 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAATTTGTCGGTTTGGTTGCCGATAGTGTGCAGATGAAG
 GGAATCAATCAATATATTATCTTTTATAAACCTAAACCACTTCATTGCGCGATTAGGCGTCTGCACGGTCGAGG
 CGGTGCGTTCCGGCCGAGATGCCGCTGATTCCCGTCAATCAGGCGGATACGCTGATTGTCGATGGGGAAGCGGTCAGC
 CTGACGGGGCATTCCGGCAATATCTTCGCGCCCGAAGGGAATTACCGGTATCTGACTTACGGGGCGGAAAAATTGCC
 GGCGGATCGTATGCCCTCCGTGTTCAAGGCGAACCTTCAAAAGGCGAAATGCTCGCGGGCACGGCAGTGTAACGGC
 GAAGTGTGCAATTTTCATACGGAAACGGCCGTCCGTCCCGTCCAGAGGCAGGTTTGCCGCAAAAGTCGATTTCCGC
 AGCAATCTGTGGACGGCATTATCGACAGCGCGATGGTTTCATATGGGTACGCAAAATTCAAAGCCGCCATCGAT
 GGAAACGGCTTTAAGGGGACTTGGACGGAAATGGCGGGGGATGTTTCCGGAAAGTTTACGGCCCGGCCGCGGAG

25

5 GAAGTGGCGGAAAAATACAGCTATCGCCCAACAGATGCGGAAAAGGGCGGATTGCGCGTGTGTTGCCGGCAAAAAAGAG
 CAGGATGGATCCGGAGGAGGAGGATGCCAAAGCAAGAGCATCCAAACCTTTCCGCAACCCGACACATCCGTCATCAAC
 GGCCCGGACCGGCCGGTCGGCATCCCCGACCCCGCCGGAACGACGGTCGGCGGCGGCGGGCGCTCTATACCGTTGTA
 CCGCACCTGTCCCTGCCCACTGGGCGGCGCAGGATTTGCCAAAAGCCTGCAATCCTTCCGCTCGGCTGCGCCAAT
 10 TTGAAAAACCGCCAAAGGCTGGCAGGATGTGTGCGCCCAAGCCTTTCAAACCCCGTCCATTCTTTTACGGCAAAACAG
 TTTTGTGAACGCTATTTACGCCGTGGCAGGTTGCAGGCAACGGAAGCCTTGCCCGGTACGGTTACCGGCTATTACGAG
 CCGGTGCTGAAGGGCGACGACAGGCGGACGGCACAAGCCCGCTTCCCGATTACGGTATTCCCGACGATTTTATCTCC
 GTCCCCCTGCCTGCCGGTTTGGCGAGCGGAAAAGCCCTTGTCGCGCATCAGGCAGACGGGAAAAACAGCGGCACAATC
 15 TTTGAAGGAAGCCGCTTCTCCCTACCACACGCGCAACCAAATCAACGGCGGCGCGCTTGACGGCAAAGCCCCGATA
 CTCGGTTACGCCGAAGACCCCGTCGAATTTTTTTTATGCACATCCAAGGCTCGGGCCGTCTGAAAACCCCGTCCGGC
 AAATACATCCGCATCGCTATGCCGACAAAAACGAACATCCCTACGTTTCCATCGGACGCTATATGGCGGACAAAGGC
 TACCTCAAGCTCGGGCAGACCTCGATGCAGGCGCATCAAAGCCTATATGCGGCAAAATCCGCAACGCGCTCGCCGAAGTT
 TTGGGTCAAACCCAGCTATATCTTTTCCGCGAGCTTGCCGGAAGCAGCAATGACGGTCCCGTCCGGCGCACTGGGC
 20 AGCCCGTTGATGGGGGAATATGCCGCGCAGTCGACCGGCACTACATTACCTTGGGCGCGCCCTTATTTGTGCCACC
 CCCCATCCGCTTACCCGCAAAGCCCTCAACCGCCTGATTATGGCGCAGGATACCGCGAGCGCGATTAAAGCGCGGTG
 CGCGTGGATTATTTTGGGGATACGGCGACGAAGCCGGCGAACTTGCCGGCAAACAGAAAACACGGGTACGCTCTGG
 CAGCTCTACCCAACGGTATGAAGCCGAATACCGCCCGTAAAAGCTT

20 1 MASPDVKSAD TLSKPAAPVV SEKETEAKED APQAGSQQG APSAQGGQDM
 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNQTA GSQNPASSTN PSATNSGGDF GRTNVGNSVV IDGPSQNIL
 201 THCKGDS CSG NNFLDEVLQ KSEFEKLSDA DKISNYKKDG KNDGKNDKVF
 25 251 GLVADSVQMK GINQYIIFYK PKPTSFAFR RSARSRRSLP AEMPLIPVNV
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGEF
 351 SKGEMLAGTA VYNGEVLHFF TENGRPSR GRFAAKVDFG SKSVDGIIDS
 401 GDGLHMGTOK FKAAIDGNF KGTWTENGSG DVSCKFYGPA GBEVAGKYSY
 451 RPTDAEKGGF GVFAGKKEQD GSGGGGQSK SIQTFPPQDT SVINGPDRPV
 30 501 GIPDPAGTTV GGGGAVYTVV PHLSLPHWAA QDFAKSLQSF RLGCANLKNR
 551 QGWQDVCAQA FQTFVHSFQA KQFFERYFTP WQVAGNGSLA GTVTGYEYEV
 601 LKGGDRRTAQ ARFPIYGIPD DFISVPLPAG LRSKALVRI RQTGKNSGTI
 651 DNTGGTHTAD LSRFPITART TAIKGRFEGS RFLPYHTRNQ INGGALDGKA
 701 PILGYAEDPV ELFFMHIIQS GRLKTPSGKY IRIGYADKNE HPYVVSIGRYM
 35 751 ADKGYLKLQ TSMQGIKAYM RQNPQRLAEV LGQNPYSYIFF RELAGSSNDG
 801 PVGALGTPLM GEYAGAVDRH YITLGAPLFV ATAHFVTRKA LNRLIMAQDT
 851 GSAIKGAVRV DYFWGYGDEA GELAGKQKTT GYVWQLLPNG MKPEYRP*

40 AG287NZ-953
 ATGGCTAGCCCCGATGTCAAGTCGGCGGACACGCTGTCAAACCTGCCGCCCTGTGTTTCTGAAAAAGAGACAGAG
 GCAAAGGAAGATGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCCGCACAAGGCGTCAAGATATGGCGGCG
 GTTTCGGAAGAAAAATACAGGCAATGGCGGTGCGGCAGCAACGACAAACCCAAATGAAGACAGGGGCGCAAAAT
 45 GATATGCCGCAAAATGCCCGCGGATACAGATAGATTGACACCGAATCACACCCCGCTTCGAATATGCCGGCGGAAAT
 ATGGA AAAACCAAGCACCGGATGCCGGGGAATCGGAGCAGCCGCAACCAACCGGATATGGCAAATACGGCGGACGGA
 ATGCAGGGTGACGATCCGTGCGCAGGCGGGAATGCGGCAATACGGCTGCCCAAGGTACAAATCAAGCCGAAAC
 AATCAAACCGCGGTTCTCAAATCCTGCCTCTTCAACCAATCCTAGCGCCACGAATAGCGGTGGTGATTGGAAGG
 ACGAACGTGGGCAATTCTGTTGTGATTGACGGGCGTCGCAAAATATAAGCTTGACCCAGTGAAGGCGATTCTTGT
 50 AGTGGCAATAATTTCTTGGATGAAGAAGTACAGTAAATCAGAATTGAAAAATTAAGTGTAGTCAGACAAAAAAGT
 AATTACAAGAAAGATGGGAAGAATGACGGGAAGAATGATAAATTTGTCGGTTTGGTTGCCGATAGTGTGCAGATGAAG
 GGAATCAATCAATATATTATCTTTTATAAACCTAAACCCACTTCATTTGCGCGATTAGGCGTTCTGCACGGTCGAGG
 CGGTGCTTCCGGCCGAGATGCCGCTGATTCCCGTCAATCAGGCGGATACGCTGATTGTCTGATGGGGAAGCGGTCAGC
 CTGACGGGGCATTCGGCAATATCTTCCGCCCGAAGGGAATTACCGGTATCTGACTTACGGGGCGGAAAAATGCCC
 55 GCGGATCGTATGCCCTCCGTGTTCAAGGCGAACCTTCAAAGGCGCAATGCTCGCGGACAGGCGAGTGTACAACGGC
 GAAGTGCTGCATTTTATACGGAACAGCGGCTCCGTCGCGTCCCGTCCAGAGGCGAGTTTGGCGCAAAAGTCGATTTCGGC
 AGCAAAATCTGTGGACGCGATTATCGACAGCGCGATGGTTTGCATATGGGTACGCAAAATTCAAAGCCGCCATCGAT
 GGAACCGCTTTAAGGGGACTTGGACGGAATGGCGGCGGGGATGTTCCGGAAGTTTACGGCCCGGCGCGGAG
 GAAGTGGCGGAAAAATACAGCTATCGCCCAACAGATGCGGAAAAGGGCGGATTTCGGCGTGTTCGGCGCAAAAAGAG
 60 CAGGATGGATCCGGAGGAGGAGGCCACCTACAAGTGGACGAATATCACGCCAACGCGCGTTTCGCCATCGACCAT
 TTCAACACCAGCACCAACGTCCGCGGTTTTTACGGTCTGACCGGTTCCGTCGAGTTTCGACCAAGCAAAACGCGACGGT
 AAAATCGACATCACCATCCCGTTGCCAACCTGCAAGCGGTTTCGCAACACTTTACCGACACCTGAAATCAGCCGAC
 ATCTTCGATGCGCCCAATATCCGGACATCCGCTTGTTCACCAAAATTCAACTTCAACGGCAAAAAACTGTTTCC
 65 GTTGACGGCAACCTGACCATGCACGGCAAAACCGCCCCGTCAACTCAAAGCCGAAAAATTCAACTGCTACCAAAGC
 CCGATGGCGAAAAACGGAAGTTTGGCGGCGGACTTCAGCACCACCATCGACCGCACCAATGGGGCGTGGACTACCTC
 GTTAACGTTGGTATGACCAAAAGCGTCCGCATCGACATCCAAATCGAGGCGCAACCAATAAAAGCTT

51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNQTA GSQNPASSTN PSATNSGGDF GRITNVGNSVV IDGPSQNTIL
 201 THCKGSDSCG NNFLDEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKFV
 251 GLVADSVQMK GINQYIIFYK PKPTSFAFR RSARSRRSLP AEMPLIPVNO
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGE
 351 SKGEMLAGTA VYNGEVLHFFH TENGRPSPSR GRFAAKVDFG SKSVDGIIDS
 401 GDGLHMGTO FKAIDGNF KGTWTENGGG DVSGKFYGP GEEVAGKYSY
 451 RPTDAEKGGF GVFAKKEQD GSGGGGATYK VDEYHANARF AIDHFNSTN
 10 501 VGGFYGLTGS VEFDAQKRDG KIDITIPVAN LQSGSQHFTD HLKSADIFDA
 551 AQYPDIRFVS TKFNFNGKKL VSVDGNLTMH GKTAPEVLKA EKFCNYQSPM
 601 AKTEVCCGDF STTIDRTKWG VDYLNVNGMT KSVRIDIQIE AAKQ*

AG287NZ-961

15 ATGGCTAGCCCGATGTCAAGTCGGCGGACACGCTGTCAAACCTGCCGCCCTGTTGTTCTGAAAAAGAGACAGAG
 GCAAAGGAAGATGCGCCACAGGCAGGTTCTCAAGGACAGGGCGGCCATCGCACAAAGGCGGTCAAGATATGCGGGCG
 GTTTCGGAAGAAAATACAGGCAATGGCGGTGCGGCAGCAACGACAAACCAAAATGAAGACGAGGGGGCGCAAAAT
 GATATGCCGCAAAATGCCGCCGATACAGATAGTTTGACACCGAATCACACCCCGCTTCAATATGCCCGCGCGAAAT
 20 ATGGAACCAAGCACCAGGATGCCGGGGAATCGGAGCAGCGGCAACCAACCGGATATGGCAAATACGGCGGACGGA
 ATGCAGGGTGACGATCCGTCGGCAGCGGGGAAATGCCGGCAATACGGCTGCCCAAGGTACAAATCAAGCCGAAAC
 AATCAAACCCCGGTTCTCAAATCCTGCTCTTCAACCAATCTAGCGCCACGAATAGCGGTGGTGAATTTGGAAGG
 ACGAACGTGGCAATTTCTGTTGTGATTGACGGGCGCTCGCAAAATATAACGTTGACCCACTGTAAAGGCGATTCTTGT
 AGTGGCAATAATTTCTTGGATGAAGAAGTACAGCTAAATCAGAATTTGAAAAATTAAGTGTGACAGACAAATAAGT
 25 GGAATCAATCAATATATATCTTTTATAAACCTAAACCCACTTCATTGCGCGATTAGGCGTTCTGCACGGTCGAGG
 CGGTGCTTCCCGCCGAGATGCCGCTGATTCCCGTCAATCAGCGGATACGCTGATTGTGCGATGGGGAAGCGGTCAGC
 CTGACGGGCAATTCGGCAATATCTTCGCGCCGAGGGAATACCGGTATCTGACTTACGGGGCGGAAAAATTGCC
 GCGGATCGTATGCCCTCCGTGTTCAAGGCGAACCTTCAAAGGCGAAATGCTCGCGGCACGGCAGGTGTACAACGGC
 30 GAAGTGTGCAATTTTCATACGGAACCGCCGTCGTCGCCGTCAGAGGCGAGTTTGGCGCAAAAGTCGATTTCCGC
 AGCAATCTGTGGACGGCATTATCGACAGCGCGGATGTTTGCATATGGGTACGCAAAATTCAGAGCCGATTTCCGC
 GGAACCGGCTTAAAGGGGACTTGGACGGAATGGCGGCGGGGATGTTTCCGGAAGTTTACGGCCCGCGCGGAG
 GAAGTGCGGGGAAAAATACAGCTATCGCCCAACAGATCGGGAAGGGCGGATTCGGCGTGTGCGCGCAAAAGAG
 CAGGATGGATCCGGAGGAGGAGGACCAACAGCAGCAGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCC
 35 TACAACATGGCCAAAGAAATCAACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACC
 AAAAAAGACGCACTGCAGCCGATGTTGAAGCCGACGACTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTG
 ACCAAAACCGTCAATGAAAAACAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGAAATAGAAAAGTTAAACA
 ACCAAGTTAGCAGACTGATGCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCAACGCCCTTGAATAAA
 TTGGGAGAAAAATATAACGACATTTGCTGAAGAGACTAAGACAAATATCGTAAAAATGATGAAAAATTAGAAGCCGTG
 40 GCTGATACCGTCGACAAGCATGCCGAAGCATTCAACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGAC
 GAAGCCGTCAAAACCGCCAATGAAGCCAAACAGACGGCGGAAGAAACCAACAAACGTCGATGCCAAAGTAAAGCT
 GCAGAACTGCAGCAGGCAAGCCGAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAAGCCGAGCTGTGCT
 GCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAACAGTGCCGACGTG
 TACACCAGAGAAAGATCTGACAGCAATTTGTCAGAAATGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGC
 45 TTGGCTTCTGCTGAAAAATCCATTGCCGATCAGTACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGC
 AAAGAAACCCGCCAAGGCCCTGTCAGAACAGCCGCTCTCCGCTGTTTCAACCTTACAACGTGGGTGCGTTCAAT
 GTAACGGCTGAGTCGGCGCTACAAATCCGAATCGGCAGTCGCCATCGGTACCGCTTCCGCTTACCGAAAACTTT
 GCCGCCAAGCAGGCGTGGCAGTCGGCACTTCTGTCGGTTCTTCCGCGAGCTACCATGTGCGGCTCAATTACGAGTGG
 TAAAGCTT

50 1 MASPDVKSAD TSKPAAPVV SEKETEAKED APQAGSQGG APSAQQGQDM
 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG
 151 TNQAENNQTA GSQNPASSTN PSATNSGGDF GRITNVGNSVV IDGPSQNTIL
 201 THCKGSDSCG NNFLDEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKFV
 55 251 GLVADSVQMK GINQYIIFYK PKPTSFAFR RSARSRRSLP AEMPLIPVNO
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGE
 351 SKGEMLAGTA VYNGEVLHFFH TENGRPSPSR GRFAAKVDFG SKSVDGIIDS
 401 GDGLHMGTO FKAIDGNF KGTWTENGGG DVSGKFYGP GEEVAGKYSY
 451 RPTDAEKGGF GVFAKKEQD GSGGGGATND DDVKAATVA IAAAYNNGQE
 60 501 INGFKAGETI YDIDRGTIT KKDATAADVE ADDFKGLGLK KVVNTLTKTV
 551 NENKQNVDAK VKAABSEIEK LTTKLADTDA ALADTDAALD ATTNALNKLK
 601 ENITFAEET KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNTKAD
 651 EAVKTANEAK QTAETKQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA
 701 VAAKVTDIKA DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK
 65 751 LDTRLASAEK SIADHDTRLN GLDKTVSDLR KETRQGLAEQ AALSGLFQPY
 801 NVGRFNVTA VGGYKSES AV AIGTGFRFTE NFAAKAGVAV GTSSGSSAAY
 851 HVGVNYEW*

Example 3 – hybrids of Δ G983

Protein 983 has the following sequence:

	983	Δ G983
5	1 MRTPTPTFPK TFKPTAMALA VATTLSACLG	GGGGGTSAPD FNAGGTGIGS
	51 NSRATTAKSA AVSYAGIKNE MCKDRSMLCA	GRDDVAVTDR DAKINAPPPN
	101 LHTGDFPNPN DAYKNLINLK PAIEAGYTGR	GVEVGIVDTG ESVGSISFPE
	151 LYGRKEHGYN ENYKNYTAYM RKEAPEDGGG	KDIEASFDDE AVIETEAKPT
	201 DIRHVKEIGH IDLVSHIIGG RSVDRPAGG	IAPDATLHIM NTNDETKNEM
10	251 MVAAIRNAWV KLGERGVRIV NNSFGTTSRA	GTADLFQIAN SEEQYRQALL
	301 DYSGGDKTDE GIRLMQQSDY GNLSYHIRNK	NMLFIFSTGN DAQAQPNNTYA
	351 LLPFYEKDAQ KGIITVAGVD RSGEKFKREM	YGEPTGTEPLE YGSNHCGITA
	401 MWCLSAPYEA SVRFTRTNPI QIAGTSFSAP	IVTGTAALLL QKYPWMSNDN
	451 LRTTLLTTAQ DIGAVGVDSK FGWGLLDAGK	AMNGPASFPF GDFTADTKGT
15	501 SDIAYSFRND ISGTGGLIKK GGSQQLQHG	NTYTGKTIIE GGSVLVLYGNN
	551 KSDMRVETKG ALIYNGAASG GSLNSDGIVY	LADTDQSGAN ETVHIKGSQ
	601 LDGKGTLYTR LGKLLKVDGT AIIGGKLYMS	ARGKGAGYLN STGRRVPFLS
	651 AAKIGQDYSF FTNIETDGGG LASLDSVEKT	AGSEGDLSY YVRRGNAART
	701 ASAAAHSAPA GLKHAVEQGG SNLENLMVEL	DASESSATPE TVETAAADRT
20	751 DMPGIRPYGA TFRAAAAVQH ANAADGVRI	NSLAATVYAD STAAHADMQG
	801 RRLKAVSDGL DHNGTGLRVI AQTQQDGGTW	EQGGVEGKMR GSTQTVGIAA
	851 KTGENTTAAA TLGMGRSTWS ENSANAKTDS	ISLFAGIRHD AGDIGYLKGL
	901 FSYGRYKNSI SRSTGADEHA EGSVNGTLMQ	LGALGGVNVP FAATGDLTVE
	951 GGLRYDLLKQ DAFAEKGSAL GWSGNSLTEG	TLVGLAGLKL SQPLSDKAVL
25	1001 FATAGVERDL NGRDVTVTGG FTGATAATGK	TGARNMPHTR LVAGLGADVE
	1051 FGNWNGLAR YSYAGSKQYG NHSGRVGVGY	RF*

Δ G983 thus has the following basic sequence:

		TSAPD FNAGGTGIGS
30	NSRATTAKSA AVSYAGIKNE MCKDRSMLCA	GRDDVAVTDR DAKINAPPPN
	LHTGDFPNPN DAYKNLINLK PAIEAGYTGR	GVEVGIVDTG ESVGSISFPE
	LYGRKEHGYN ENYKNYTAYM RKEAPEDGGG	KDIEASFDDE AVIETEAKPT
	DIRHVKEIGH IDLVSHIIGG RSVDRPAGG	IAPDATLHIM NTNDETKNEM
	MVAAIRNAWV KLGERGVRIV NNSFGTTSRA	GTADLFQIAN SEEQYRQALL
35	DYSGGDKTDE GIRLMQQSDY GNLSYHIRNK	NMLFIFSTGN DAQAQPNNTYA
	LLPFYEKDAQ KGIITVAGVD RSGEKFKREM	YGEPTGTEPLE YGSNHCGITA
	MWCLSAPYEA SVRFTRTNPI QIAGTSFSAP	IVTGTAALLL QKYPWMSNDN
	LRTTLLTTAQ DIGAVGVDSK FGWGLLDAGK	AMNGPASFPF GDFTADTKGT
	SDIAYSFRND ISGTGGLIKK GGSQQLQHG	NTYTGKTIIE GGSVLVLYGNN
40	KSDMRVETKG ALIYNGAASG GSLNSDGIVY	LADTDQSGAN ETVHIKGSQ
	LDGKGTLYTR LGKLLKVDGT AIIGGKLYMS	ARGKGAGYLN STGRRVPFLS
	AAKIGQDYSF FTNIETDGGG LASLDSVEKT	AGSEGDLSY YVRRGNAART
	ASAAAHSAPA GLKHAVEQGG SNLENLMVEL	DASESSATPE TVETAAADRT
	DMPGIRPYGA TFRAAAAVQH ANAADGVRI	NSLAATVYAD STAAHADMQG
45	RRLKAVSDGL DHNGTGLRVI AQTQQDGGTW	EQGGVEGKMR GSTQTVGIAA
	KTGENTTAAA TLGMGRSTWS ENSANAKTDS	ISLFAGIRHD AGDIGYLKGL
	FSYGRYKNSI SRSTGADEHA EGSVNGTLMQ	LGALGGVNVP FAATGDLTVE
	GGLRYDLLKQ DAFAEKGSAL GWSGNSLTEG	TLVGLAGLKL SQPLSDKAVL
50	FATAGVERDL NGRDVTVTGG FTGATAATGK	TGARNMPHTR LVAGLGADVE
	FGNWNGLAR YSYAGSKQYG NHSGRVGVGY	RF*

Δ G983 was expressed as a hybrid, with ORF46.1, 741, 961 or 961c at its C-terminus:

Δ G983-ORF46.1

55	ATGACTTCTGCGCCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCA
	GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAGACAGAACATGCTCTGTGCCGGTCGGGATGACGTTGCG
	GTTACAGACAGGATGCCAAAATCAATGCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCA
	TACAAGAATTGATCAACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGAC
	ACAGGCGAATCCGTCGGCAGCATATCCTTTCCCGAACTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAA
	AACTATACGGCTATATGCGGAAGGAAGCGCCTGAAGACGGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAG
60	GCCGTTATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAGAAATCGGACACATCGATTGGTCTCCCAT

ATTATTGGCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACG
 AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACCTGGCGTGCGC
 ATCGTCAATAACAGTTTGTGAACAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTTCGGAGGAGCAG
 TACCGCCAAGCGTTGCTCGACTATTCCGGCGGTGATAAAACAGACGAGGGTATCCGCCGTGATGCAACAGAGCGATTAC
 5 GGCAACCTGTCTTACCACATCCGTAATAAAAAATGCTTTTCATCTTTTCGACAGGCAATGCAGCCACAAGCTCAGCCC
 AACACATATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCGAGGCGTAGACCGCAGT
 GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTCGCGAATT
 ACTGCCATGTGGTGCCTGTCGGCACCTATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTGCCGGA
 10 ACATCCTTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAAC
 CTGCGTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGCGCTGGACAGCAAGTTCCGGCTGGGGACTGCTG
 GATGCGGTAAAGCCATGAACGGACCCGCTTTCCTGCTCGGCGACTTTACCGCGGATACGAAAGGTACATCCGAT
 ATTGCCTACTCCTTCCGTAACGACATTTTCAGGCACGGGCGGCTGATCAAAAAAGGCGGCAACCTGCAACTGCAC
 GGCAACAACACCTATACGGGCAAAACCATTTATCGAAGGCGGTTTCGCTGGTGTGTACGGCAACAACAAATCGGATATG
 15 CGCGTCGAAACCAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTGTCTAT
 CTGGCAGATACCGACCAATCCGGCGCAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAGGTACG
 CTGTACACACGTTTGGGCAAACTGCTGAAAGTGGACGGTACGGCGATTATCGGCGGCAAGCTGTACATGTGGGCACGC
 GGCAAGGGGCGAGGCTATCTCAACAGTACCGGACGAGCTGTTCCCTTCTGAGTGCAGCGCAAAATCGGGCAGGATTAT
 TCTTTCTTCAAAACATCGAAACCGAGCGGCGCTTGGCTTCCCTCGACAGCGTCAAAAAACAGCGGCGAGTGAA
 20 GCGGACACGCTGTCTATTATGTCGCTCGCGGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTCCGCGCCCGCC
 GGTCTGAAACACGCGTAGAACAGGGCGGCAGCAATCTGGAACCTGATGGTCAAGTGGATGCCTCCGAATCATCC
 GCAACACCCGAGACGGTTGAAACTGCGGCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTC
 CGCGCAGCGGCAGCCGTACAGCATGCGAATGCCGCGCAGCGGTGTACGCATCTTCAACAGTCTCGCGGCTACCGTCTAT
 GCGACATACCGCCCGCATGCGGATATGCGAGGACGCGGCTGAAAGCGGTATCGGACGGTTGGACCACAACGGC
 25 ACGGGTCTGCGCGTCACTCGCGCAACCCCAACAGGACGGTGGAAACGTCGGGAAACAGGGCGAAATGCGC
 GGCAGTACCCAAACCGTCCGCTTGCCTGCGGCAAAACCGGCAAAATACGACAGCAGCGGCCACACTGGGCATGGGACGC
 AGCACATGGAGCGAAACAGTGCAAATGCAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCAGATGCGGGC
 GATATCGGCTATCTCAAAGGCTGTCTCTTACGACGCTACAAAAACAGCATCAGCCCGCAGCACCGGTGCGGACGAA
 CATGCGGAAGGCAGCGTCAACGGCAGCGTGTATGCGAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGCCTGCAACG
 30 GGATTTGACGGTCAAGGCGGCTGCGCTACGACCTGCTCAAAACAGGATGCATTGCGCGAAAGGAGGAGTGTCTT
 GGCTGGAGCGGCAACAGCCTCACTGAAGGACGCTGGTGGAGCTGCGGGCTGTAAGCTGTGCGAACCTTGAAGCGAT
 AAAGCCGCTCTGTTTGAACGGCGGGCGTGGAAACGCGACCTGAACGGACCGGACTACAGGTAACGGGCGGCTTTACC
 GCGCGACTGCAGCAACCGGCAAGACGGGGGACGCAATATGCCGACACCCGCTGCTGTTGCCGGCTGGGCGCGGAT
 GTCGAATTCGGCAACCGCTGGAACGGCTTGGCAGCTTACAGCTACGCGGTTCCAAACAGTACGGCAACCAACAGCGGA
 35 CGAGTCGGCGTAGGCTACCGGTTCTCGACGGTGGCGGAGGCACTGGATCCTCAGATTGGGAAACGATTCTTTTATC
 CCGAGGTTCTCGACCGTCAGCATTTGCAACCCGACGGGAAATACCACTATTTCGGCAGCAGGGGGAACTTCCCGAG
 CGCAGCGGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTGATGATTCAACAGGCGGCCATTAAA
 GGAATATCGGCTACATTGTCCGCTTTTCCGATCAGGGCACGAAGTCCATTCCCCCTTCGACAACCATGCCTCACAT
 TCCGATTCTGATGAAGCGGTAGTCCCGTTGACGGATTTAGCTTTTACCGCATCCATTGGGACGGATACGAACACCAT
 40 CCCGCCGACGGCTATGACGGGCCACAGGGCGGCGGCTATCCCGCTCCCAAAGGCGCGAGGGATATATACAGCTACGAC
 ATAAAAGGCGTTGCCCAAAATATCCGCTCAACCTGACCGACAACCGCAGCAGCCGCAACCGCTTGGCGACCGTTTC
 CACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAAACGCGCCACCCGATACAGCCCGAGCTGGAC
 AGATCGGGCAATGCCGCGGAAGCCTTCAACGGCACTGCAGATATCGTTAAAAACATCATCGGCGCGGAGAGAAATT
 GTCGGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATTGCTGTATGCACGGCTTGGGTCTGCTTTCC
 45 ACCGAAACAAGATGGCGCGCATCAACGATTGTCAGATATGGCGCAACTCAAAGACTATGCCGACGAGCCATCCGC
 GATTGGGCGAGTCCAAACCCCAATGCCGCACAAGGCATAGAAGCCGTCAGCAATATCTTTATGGCAGCCATCCCCATC
 AAGGGATTTGGAGCTGTTCCGGGAAAAATACGGCTTGGGCGGCATCAGGCACATCCTATCAAGCGGTGCGAGATGGGC
 CGGATCGATTGCGGAAAGGAAATCCGCGTCAAGGACAAATTTGCGGATGCGGCAAGCCAAATACCCGCTCCCT
 TACCATTCCCGAAATATCCGTTCAAACCTTGGAGCAGCGTTACGGCAAGAAAAACATCACTCTCAACCGTCCCGCG
 50 TCAAACGGCAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTACCGTTTGAAGGTAAGGGTTTCCG
 AATTTTGAGAAGCACGTGAAATATGATACGCTCGAGCACCACCACCACCACTGA

1 MTSAPDFNAG GTGIGSNSRA TTAASAASVY AGIKNEMCKD RSMLCAGRDD
 51 VAVTDRDAKI NAPPPNLHTG DFPNPNDAYK NLINLKPAIE AGYTGRGVEV
 101 GIVDTGESVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGGKDIE
 155 151 ASFDDEAVIE TEAKPTDIRH VKEIGHIDL V SHIIGGRSVD GRPAGGIAPD
 201 ATLHIMNTND ETKNEMMVAA IRNAWVKLGE RGVRIVNNNSF GTTSRAGTAD
 251 LFQIANSEEQ YRQALLDYSG GDKTDEGIRL MQQSDYGNLS YHIRNKNMFL
 301 IFSTGNDAQA QPNTYALLPF YEKDAQKGI TVAGVDRSGE KFKREMYGEP
 351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG
 60 401 TAALLLQKYP WMSNDNLRTT LLTTAQDIGA VGVDKSGFWG LLDAGKAMNG
 451 PASFPFGDFT ADTKGTSDIA YSFRNDISGT GGLIKKGGSQ LQLHGNNTYT
 501 GKTIIEGGSL VLYGNKSDM RVETKGALIV NGAASGGSLN SDGIVYLADT
 551 DQSGANETVH IKGSLQLDGK GTLYTRLGKL LKVDGTAIIG GKLYMSARGK
 601 GAGYLNSTGR RVPFLSAAKI GDYSFFFTNI ETDGGLLASL DSVEKTAGSE
 65 651 GDTLSYYVRR GNAARTASAA AHSAPAGLKH AVEQGSNLE NLMVELDASE
 701 SSATPETVET AAADRTDMPG IRPYGATFRA AAQVQHANA DGVRIFFNSLA
 751 ATVYADSTAA HADMQRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG

-15-

801 VEGKMRGSTQ TVGLIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDSISLF
 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMQLGAL
 901 GGVNVPFAAT GDLTVEGLR YDLLKQDAFA EKGSALGWSG NSLTEGTLVG
 951 LAGLKLSQL SDKAVLFATA GVERDLNGRD YTVTGGFTGA TAATGKTGAR
 1001 NMPHTRLVAG LGADVEFGNG WNGLARYSYA GSKQYGNHSG RVGVGYRFLD
 1051 GGGGTGSSDL ANDSFIRQVL DRQHFEPDGK YHLFGSRGEL AERSGHIGLG
 1101 KIQSHQLGNL MIQQAIAKGN IGYIVRFS DH GHEVHSPFDN HASHSDSDEA
 1151 GSPVDGFSLY RIHWDGYEHH PADGYDGPQG GGYPAKPGAR DIYSYDIKGV
 1201 AQNIRLNLTD NRSTGQRLAD RFHNAGSMLT QGVGDGFKRA TRYSPELDRS
 1251 GNAAEAFNGT ADIVKNIIGA AGEIVGAGDA VQGISSEGSNI AVMHGLGLLS
 1301 TENKMARIND LADMAQLKDY AAAAIRDWAV QNPNAAQGIE AVSNIFMAAI
 1351 PIKIGIAVRG KYGLGGITAH PIKRSQMGAI ALPKGKSAVS DNFADAAYAK
 1401 YPSPYHSRNI RSNLEQRYGK ENITSSTVPP SNGKNVKLAD QRHPKTGVFPF
 1451 DGKGFNPFKE HVKYDTLEHH HHHH*

AG983-741

ATGACTTCTGCGCCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCA
 GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAGACAGAACGATGCTCTGTGCCGGTCCGGATGACGTTGCCG
 GTTACAGACAGGGATGCCAAAATCAATGCCCCCCCCCCGAAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCA
 TACAAGAATTTGATCAACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGAC
 ACAGGCGAATCCGTCGGCAGCATATCCTTTCCCGAACTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAA
 AACTATACGGCGTATATGCGGAAGGAAGCGCCTGAAGACGGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAG
 GCCGTATAGAGACTGAAGCAAGGCCGACGGATATCCGCCACGTAAAGAAATCGGACACATCGATTGGTCTCCCAT
 ATTATTGGCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACG
 AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAAGCTGGCGTGC GC
 ATCGTCAATAACAGTTTGGGAACAACATCGAGGCGAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAG
 TACCGCCAAGCGTTGCTCGACTATTCCGGCGGTGATAAAACAGACGAGGATATCCGCCTGATGCAACAGAGCGATTAC
 GGCAACCTGTCTTACCACATCCGTAATAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCC
 AACACATATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGACGGCGTAGACCGCAGT
 GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACC GGGTACAGAACCGCTTGAGTATGGCTTCAACCATTCGCGAAT
 ACTGCCATTTGGTGCTGTGCGGACCCATGAAGCAAGCGTCCGTTTCAACCGTACAAACCCGATTCAAATTCGCGGA
 ACATCCTTTTCCGCAACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAAC
 CTGCGTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCCGCTGGGACTGCTG
 GATGCGGGTAAGGCCATGAACGACCCGCGTCTTTCCGTTTCGGCGACTTTTACCGCCGATACGAAAGGTACATCCGAT
 ATTGCCCTACTCCTTCCGTAACGACATTTTACGACGCGGCGGCTGATCAAAAAGCGCGCAGCAACTGCAACTGCAC
 GGCAACAACACCTATACGGGCAAAACCATTTTCAAGGCGGTTCGCTGCTGTTGTGACGGCAACAACCAATTCGATATG
 CGCGTCGAAACCAAGGTGCGCTGATTTTATAACGGGCGGCATCCGGCGGCAGCTGAACAGCGACGGCATTGTCTAT
 CTGGCAGATACCGACCAATCCGGCGCAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAGGTACG
 CTGTACACACGTTTGGGCAAACTGCTGAAAGTGGACGGTACGGCGATTATCGGGCGCAAGCTGTACATGTCCGCACGC
 GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTTTCCCTTCTGAGTGCCGCCAAATTCGGGCAAGGATTAT
 TCTTTCTTCAAAACATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCTCGAAACCAACCGGGCAGTGAA
 GGCAGACGCTGTCTATTATGTCGCTCGCGCAATGCGGCACGGACTGCTTCCGCAACGACATTCGCGCCCCGCC
 GGTCTGAAACACGCGCTAGAACAGGGCGGCAATCTGGAACCTGATGGTGAAGTGGATGCTCCGAATCATCC
 GCAACACCCGAGACGGTTGAAACTGCGGCAGCGACCGCACAGATATGCCGGGCATCCGCCCCACGGCGCAACTTTC
 CGCGCAGCGGACGCTACAGCATGCGAATGCCGCCGACGTTGTACGATCTTCAACAGTCTCGCCGCTACCGTCTAT
 GCCGACAGTACCGCCGCCATGCGGATATGCAGGACGCGCGCTGAAAGCCGTATCGGACGGGTTGGACCACAACGGC
 ACGGGTCTGCGCGTATCGCGCAAAACCAACAGGACGGTGGAAACGTGGAAACAGGGCGGTGTTGAAGGCAAAATGCGC
 GGCAGTACCCAAACCGTCGGCATTCGCCGAAACCGCGGCAAAATACGACAGCAGCGCCACACTGGGCATGGGACGC
 AGCAGATGGAGCGAAACAGTGCAATGCAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCACGATGCGGGC
 GATATCGGCTATCTCAAAGGCTGTTCTCTTACGGACGCTACAAAACAGCATCAGCCCGCAGCACCGGTGCGGACGAA
 CATGCGGAAGGCAGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGGCCGCAACG
 GGAGATTTGACGGTCAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTGCGCGAAACAGGCAAGTCTTGG
 GGCTGGAGCGGCAACAGCCTCACTGAAGGCAGCTGGTGCAGCTCGCGGGTCTGAAGCTGTGCGAACCCCTTGAGCGAT
 AAAGCCGTCCTGTTTGAACGGCGGGCGTGAACCGCGACCTGAACGGACGCGACTACACGGTAACGGGCGGCTTTACC
 GGCGGACTGCAGCAACCGGCAAGACGGGGCACGCAATATGCCGCACACCCGCTGTTGTCGGGCTGGGCGCGGAT
 GTCGAATTCGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGGTTCCAAACAGTACGGCAACACAGCGGA
 CGAGTCGGCTAGGCTACCGGTTCTTCGAGGGATCCGGAGGGGGTGGTGTGCGCCCGACATCGGTGCGGGGCTTGGC
 GATGCACTAACCGCACCGCTCGACCATAAAGACAAAGGTTTGCAGTCTTTGACGCTGGATCAGTCCGTGAGAAAAAC
 GAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGAACCGGTGACAGCTCAATACGGGCAAAATGAAG
 AACGACAAGTTCAGCCGTTTCGACTTTATCCGCCAAATCGAATGAGTGGACGGGCGAGCTCATTACCTTGGAGAGTGGAGAG
 TTCCAAGTATACAAACAAAGCCATTCCGCTTAACCGCTTTTACAGCCGAGCAATACAAGATTTCGGAGCATTCGGGG
 AAGATGGTTGCGAAACGCCAGTTTCAAGATCGGCACATAGCGGGCAACATACATCTTTTGACAAGCTTCCCGAAGGC
 GGCAGGGGACATATCGCGGGACGGCGTTCCGTTTCAGACGATGCCGGCGGAAACTGACCTACACCATAGATTTCGCC
 GCCAAGCAGGGAACGGCAAAATCGAACATTTGAAATCGCCAGAACTCAATGTGCACCTGGCCGCCCGCATATCAAG
 CCGGATGGAAACGCCATCCGTCATCAGCGGTTCCGTCCTTTACAACCAAGCCGAGAAAGGCAGTTACTCCCTCGGT

ATCTTTGGCGGAAAAGCCCAGGAAGTTGCCGGCAGCGCGGAAGTGAAAACCGTAAACGGCATACGCCATATCGGCCTT
GCCGCCAAGCAACTCGAGCACCACCACCACCACCCTGA

5	1	MTSAPDFNAG	GTGIGSNSRA	TTAKSAAVS	AGIKNEMCKD	RSMLCAGRDD
	51	VAVTDRDAKI	NAPPPNLHTG	DFPNPNDAYK	NLINLKPAIE	AGYTGRGVEV
	101	GIVDTGESVG	SISFPELYGR	KEHGYNENYK	NYTAYMRKEA	PEDGGGKDIE
	151	ASFDDEAVIE	TEAKPTDIRH	VKEIGHIDL	SHIIGGRSVD	GRPAGGIAPD
	201	ATLHIMNTND	ETKNEMMVAA	IRNAWVKLGE	RGVRIVNNSF	GTTSRAGTAD
10	251	LFQIANSEEQ	YRQALLDYS	GDKTDEGIRL	MQQSDYGNLS	YHIRNKNMLF
	301	IFSTGNDQA	QPNTYALLPF	YEKDAQKGI	TVAGVDRSGE	KFKREMYGEP
	351	GTEPLEYGSN	HCGITAMWCL	SAPYRASVRF	TRTNPIQIAG	TSFSAPIVTG
	401	TAALLLQKYP	WMSNDNLRTT	LLTTAQDIGA	VGVDKFGWG	LLDAGKAMNG
	451	PASFPFGDFT	ADTKGTSDIA	YSFRNDISGT	GGLIKKGGSQ	LQLHGNNTYT
15	501	GKTIIEGGS	VLYGNNSDM	RVETKGALY	NGAASGGS	SDGIVYLADT
	551	DQSGANETVH	IKGSLQLDGK	GTLYTRLGKL	LKVDGTAIIG	GKLYMSARGK
	601	GAGYLNSTGR	RVPFLSAAKI	GQDYSFFTNI	ETDGGLLASL	DSVEKTAGSE
	651	GDTLSYYVRR	GNAARTASAA	AHSAPAGLKH	AVEQGGSNLE	NLMVELDASE
	701	SSATPETVET	AAADRTDMPG	IRPYGATFRA	AAAVQHANA	DGVRIFNLSA
20	751	ATVYADSTAA	HADMQRRLK	AVSDGLDHNG	TGLRVIAQTQ	QDGGTWEQGG
	801	VEGKMRGSTQ	TVGIAAKTGE	NTTAAATLGM	GRSTWSENSA	NAKTDSISLF
	851	AGIRHDAGDI	GYLKGLFSYG	RYKNSISRST	GADEHAEGSV	NGTLMQLGAL
	901	GGVNVFFAAT	GDLTVEGGLR	YDLLKQDAFA	EKGSALGWSG	NSLTEGLTVG
	951	LAGLKLSQLP	SDKAVLFATA	GVERDLNGRD	YTVTGGFTGA	TAATGKTGAR
25	1001	NMPHTRLVAG	LGADVEFGNG	WNGLARYSYA	GSKQYGNHSG	RVGVGYRFLF
	1051	SGGGGGVAAD	IGAGLADALT	APLDHKDKGL	QSLTLDQSVR	KNEKLKLAAQ
	1101	GAEKTYGNGD	SLNTGKLKND	KVSRFDFIRQ	IEVDGQLITL	ESGEFQVYKQ
	1151	SHSALTAFQT	EQIQDSEHSG	KMVAKRQFRI	GDIAGEHTSF	DKLPEGGRAT
	1201	YRGTAFGSDD	AGGKLYTID	FAAKQGNKGI	EHLKSPELNV	DLAAADIKPD
30	1251	GKRHAIVSGS	VLYNQAEKGS	YSLGIFGGKA	QEVAGSAEVK	TVNGIRHIGL
	1301	AAKQLEHHHH	HH*			

AG983-961

35	ATGACTTCTGCGCCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCA
	GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGATGACGTTGCG
	GTTACAGACAGGGATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCA
	TACAAGAAATTTGATCAACCTCAAACTGCAATTGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTTCGAC
	ACAGGCGAATCCGTCGGCAGCATATCCTTTCCCGAACTGTATGGCAGAAAAGAACACCGCTATAACGAAAATTACAAA
40	AACATATACGGCGTATATGCGGAAGGAGCGCCTGAAGACGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAG
	GCCGTTATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATTCGATTGGTCTCCCAT
	ATTATTTGGCGGGCGTTCGTTGGACGGCAGACCTGCAGGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACG
	AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTGGCGTGGCG
	ATCGTCAATAACAGTTTGTGAACAACATCGAGGCGAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAG
45	TACCGCCAAGCGTTGCTCGACTATTCCGGCGGTGATAAACAGACGAGGGTATCCGCCGTGATGCAACAGAGCGATTAC
	GGCAACCTGTCTTACCACATCCGTAATAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCAACAGCTCAGCCC
	AACACATATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGAGGCGTAGACCGCAGT
	GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACC GGGTACAGAACCCTTGAGTATGGCTCCAACCATTTGCGGAATT
	ACTGCCATGTGGTGCTGTTCGGCACCCCTATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTTGCCGGA
50	ACATCCTTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCGTGATGAGCAACGACAAC
	CTGCGTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCCGCGTGGACAGCAAGTTCCGGCTGGGGACTGCTG
	GATGCGGGTAAGGCATGAACGGACCCGCGTCTTTCCGTTCCGGCACTTTACCGCGGATACGAAAGGTACATCCGAT
	ATTGCTACTCCTTCCGTAACGACATTTTCAGGCACGGGCGGCTGATCAAAAAGGCGGCAGCAACTGCAACTGCAC
	GGCAACAACACCTATACGGGCAAAACCATTTATCGAAGGCGGTTCGCTGGTGTGTACGGCAACAACAAATCGGATATG
55	CGCGTCGAAACCAAAGGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTGTCTAT
	CTGGCAGATACCGACCAATCCGGCGCAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAGGTACG
	CTGTACACAGCTTTGGGCAAACCTGCTGAAAGTGACGGTACGGCGATTATCGGGCGGCAAGCTGTACATGTCCGCACGC
	GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGAGCTGTTCCCTTCTGAGTGGCGCCAAAATCGGGCAGGATTAT
	TCTTTCTTTCACAAACATCGAAACCGACGGCGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGA
60	GGCGACACGCTGTCTATTATGTCCGTCGCGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTCCGCGCCGCC
	GGTCTGAAACACGCCGTAGAACAGGGCGGCAGCAATCTGCAAAACCTGATGCTTCGAACTCGAATCGCTCCGAATCAAC

-17-

CATGCCGAAGGCAGCGTCAACGGCAGCGTGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTCCGCGCAACG
 GGAGATTTGACGGTGAAGGGCGTCTGCGCTACGACCTGCTCAAACAGGATGCATTGCGCCGAAAAAGGCAGTGTCTTG
 GGCTGGAGCGGCAACAGCCTCACTGAAGGCAGCGTGGTGGACTCGCGGGTCTGAAGCTGTGCAACCCCTTGAGCGAT
 5 AAAGCCGTCCTGTTTGAACGGCGGGCGTGAACGCGACCTGAACGGACGCGACTACACGGTAACGGGCGGCTTTACC
 GGCAGCGACTGCAGCAACCGGCAAGACGGGGGCACGCAATATGCCGCACACCCGCTGTGGTTGCCGGCCTGGGCGCGGAT
 GTCGAATTCGGCAACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGGTTCCAAACAGTACGGCAACCACAGCGGA
 CGAGTCGGCGTAGGCTACCGGTTCTCGAGGGTGGCGGAGGCACTGGATCCGCCACAAACGACGACGATGTTAAAAAA
 GCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAAGAAATCAACGGTTTCAAAGCTGGAGAGACCATCTAC
 10 GACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGACTTTAAAGGTCTG
 GGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAACAAACAAACGTCGATGCCAAAGTAAAGCT
 GCAGAACTGTAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATACTGATGCCGCTCTG
 GATGCAACCAACACGCTTGAATAAATTTGGGAGAAAAATAACGACATTTGCTGAAGAGACTAAGACAAATATCGTA
 AAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTCAACGATATCGCCGATTCA
 15 TTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCAAATGAAGCCAAACAGACGGCCGAAGAAACCAAA
 CAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAGCCGAAGCTGCCGCTGGCACAGCTAATACT
 GCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAAGCTGATATCGCTACGAACAAAGATAATATT
 GCTAAAAAAGCAACAGTGCAGCGCTGTACACGAGAGAAGAGTCTGACAGCAAAATTTGTGAGAATTGATGGTCTGAAC
 GCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATCACGATACTCGCCTGAACGGT
 20 TTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCGCCAAAGCCCTTGCAAGCAAGCCGCGCTCTCCGGCTGTGTT
 CAACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCGGCGGCTACAAATCCGAATCGGCAGTCGCCATCGGT
 ACCGGCTTCCGCTTTACCGAAAACTTTGCCGCCAAAGCAGCGTGGCAGTCGGCACTTCGTCCGGTTCTTCCGCAGCC
 TACCATGTCGGCGTCAATTACGAGTGGCTCGAGCACCACCACCACCACCTGA

1 MTSAPDFNAG GTGIGSNSRA TTAKSAAVSY AGIKNEMCKD RSMLCAGRDD
 25 51 VAVTDRDAKI NAPPNNLHTG DFPNPNDAYK NLINLKPAIE AGYTRGRVEV
 101 GIVDTGESVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGGKDIE
 151 ASFDDEAVIE TEAKPTDIRH VKEIGHIDL V SHIIGRSVD GRPAGGIAPD
 201 ATLHIMNTND ETKNEMMVA IRNAWVKLGE RGVRIVNSF GTTSRAGTAD
 251 LFQIANSEEQ YRQALLDYS GDKTDEGIRL MQQSDYGNLS YHIRNKNMLF
 30 301 IFSTGNDQA QPNTYALLPF YEKDAQKGI TVAGVDRSGE KFKREMYGEP
 351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG
 401 TAALLLQKYP WMSNDNLRTT LLTTAQDIGA VGVDSKFGWG LLDAGKAMNG
 451 PASFPFGDFT ADTKGTSDIA YSFRNDISGT GGLIKKGGSQ LQLHGNNTYT
 501 GKTIEGGS VLYGNKSDM RVETRGALIY NGAASGGS LN SDGIVYLADT
 35 551 DQSGANETVH IKGSLQLDGK GTLYTRLGKL LKVDGTAIIG GKLYMSARGK
 601 GAGYLNSTGR RVPFLSAKI GDYSFFTNI ETDGGLLASL DSVEKTAGSE
 651 GDLSYYVRR GNAARTASAA AHSAPAGLKH AVEQGSNLE NLMVELDASE
 701 SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANA DGVRIFNSLA
 751 ATVYADSTAA HADMQRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG
 40 801 VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDSISLF
 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMQLGAL
 901 GGVNVPFAAT GDLTVEGGLR YDLLKQDAFA EKGSALGWSG NSLTEGLTVG
 951 LAGLKLSQPL SDKAVLFATA GVERDLNDRD YTVTGFTGA TAATGKTGAR
 1001 NMPHTRLVAG LGADVEFGNG WNGLARYSYA GSKQYGNHSG RVGVGYRFLF
 45 1051 GGGGTGSATN DDDVKKAATV AIAAAYNNGQ EINGFKAGET IYDIDEDGTI
 1101 TKKDATAADV EADDFKGLGL KKVVTNLTKT VLENKQNVDA KVKAASEIE
 1151 KLTTKLADTD AALADTDAAL DATTNALNKL GENITTFEE TKTNIWKIDE
 1201 KLEAVADTVD KHARAFNDIA DSLDETNTKA DEAVKTANEA KQTAETKQN
 1251 VDAKVKAET AAGKAEAAAG TANTAADKAE AVAAKVTDIK ADIATNKDNI
 50 1301 AKKANSADV TREESDSKFV RIDGLNATTE KLDTRLASAE KSIADHDTRL
 1351 NGLDKTVSDL RKETROGLAE QAALSGLFQP YNVGRFNVTA AVGGYKSESA
 1401 VAIGTGFRFT ENFAAKAGVA VGTSSGSAA YHVG VNYEWL EHHHHHH*

55 AG983-961c
 ATGACTTCTGCGCCGACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACACAGCGAAATCAGCA
 GCAGTATCTTACGCCGGTATCAAGAACGAAATGTGCAAAGACAGAAGCATGCTCTGTGCCGGTGGGATGACGTTGCG
 GTTACAGACAGGGATGCCAAATCAATGCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCA
 60 TACAAGAATTTGATCAACCTCAAACCTCAATGAAGCAGGCTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGAC
 ACAGGCGAATCCGTCGGCAGCATATCCTTTCCCGAACTGTATGGCAGAAAAGAACACGGCTATAACGAAAATTACAAA
 AACTATACGGCGTATATGCGGAAGGAAGCGCGTGAAGACGGCGGTAAGACATTGAAGCTTCTTTCCGACGATGAG
 GCCGTATAGAGACTGAAGCAAAAGCCGACGATATCCGCCACGTAAAAGAAATCGGACACATCGATTGGTCTCCCAT
 ATTATTGGCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATTCGCCCGATGCGACGCTACACATAATGAATACG
 AATGATGAAACCAAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTGGCGTGGCG
 65 ATCGTCAATAACAGTTTTTGGAAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAG
 TACCGCCAAGCGTTGCTCGACTATTCCGGCGGTGATAAAACAGACGAGGGTATCCGCCTGATGCAACAGAGCGATTAC
 GGCAACCTGTCTTACCACATCCGTAATAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCC

AACACATATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCGAGGCGTAGACCGCAGT
 GGAGAAAAGTTCAAACGGGAAATGTATGGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTTGCCGAATT
 ACTGCCATGTGGTGCCTGTTCGGCACCCTATGAAGCAAGCGTCCGTTTACCCGTACAAAACCCGATTCAAATTTGCCGGA
 5 ACATCCTTTTCCGCACCCATCGTAACCGGCACGGCGGCTCTGCTGTCAGAAAATACCCGTGGATGAGCAACGACAAC
 CTGCGTACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCCGGCTGGGGACTGCTG
 GATGCGGGTAAGGCCATGAACGGACCCGCTCCTTTCCGTTCCGGCACTTTACCGCCGATACGAAAAGGTACATCCGAT
 ATTGCCTACTCCTTCCGTAAACGACATTTAGGCACGGCGCGCTGATCAAAAAAGGGCGAGCAACTGCAACTGCAC
 10 GGCAACAACACCTATACGGGCAAAACCATTTATCGAAGGCGGTTCCGTTGGTGTGTACGGCAACAACAAATCGGATATG
 CGCGTCGAAACCAAAGGTGCGCTGATTATTAACGGGCGGCATCCGGCGGCAGCTGAACAGCGACGGCATTTGTCTAT
 CTGGCAGATACCGACCAATCCGGCGCAAAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAGGTACG
 CTGTACACACGTTTGGGCAAACTGCTGAAAGTGGACGGTACGGCGATTATCGCGCGCAAGCTGTACATGTCGGCACGC
 GGCAAGGGGGCAGGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCTGAGTGCAGCGCAAAATCGGGCAGGATTAT
 TCTTTCTTCAAAACATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAAAGCGCGGAGTGA
 15 GGCGACACGCTGTCTTATGTCGCGCGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTTCCGCGCCCGCC
 GGTCTGAAACACGCGTAGAACAGGCGCGCAATTCGAAACCTGATGGTTCGAACTGGATGCTTCCGAATCATCC
 GCAACACCCGAGACGGTTGAACTGCGCGACGGCGACCGCACAGATATGCCGGGCATCCGCCCCACGCGCAACTTTTC
 CGCGCAGCGCGCAGCCGTACAGCATGCGAATGCCGCCGACGGTGTACGCATCTTCAACAGTCTCGCGCTACCGTCTAT
 CCGGACAGTACCGCCCGCCATGCCGATATGCAGGGACGCCGCTGAAAGCGTATCGGACGGGTTGGACCAACACGGC
 20 ACGGCTCTGCGCGTCATCGCGCAAAACCAACAGGACGGTGAACGTTGGGAACAGGGCGGTTGTAAGGCAAAATGCGC
 GGCAGTACCCAAACCGTCGGCATTGCCCGCAAAACCGCGCAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGC
 AGCAGATGGAGCGAAACAGTGCAAATGCAAAACCGACAGCATAGTCTGTTTGCAGGCATACGGCAGCATGCGGGC
 GATATCGGCTATCTCAAAGGCTGTTCTCTTACGGACGCTACAAAACAGCATCAGCCGACGACCCGTTGCGGACGAA
 CATGCGGAAGGCAGCGTCAACGGCAGCGTGTGAGCTGGGCGCACTGGCGGTTGTCACGTTCCGTTTGGCGCAACG
 25 GGAGATTTGACGGTTCGAAGGCGGCTGCGCTACGACCTGCTCAAACAGGATGCATTCCGCCGCAAAAGCGGCTGCTTG
 GCTGGAGCGGCAACAGCCTCACTGAAGGCAGCTGGTGGGACTCGCGGCTGTAAGCTGTGCGCAACCCCTTGAGCGAT
 AAAGCCGTCCTGTTTGAACGGCGGGCGTGAACCGGACCTGAACGGACGCGACTACAGGTAACGGGCGGCTTTACC
 GCGCGACTGCGAGCAACCGGCAAGACGGGGCGCAATATGCCGCACACCCGCTGTTGTTGCCGCGCTGGGCGCGGAT
 GTCGAATTCGGCAACGGCTGGAACGGCTTGGCAGCTTACAGCTACCGCGGTTCCAAACAGTACGGCAACACAGCGGA
 30 CGAGTCCGCGTAGGCTACCGGTTCTCTGAGGGTGGCGGAGGCACTGGATCCGCCCAAAACGACGAGATGTTAAAAA
 GCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAACGGTTTCAAAGCTGGAGACCATCTAC
 GACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCCGACGACTTTAAAGGCTCTG
 GGTCTGAAAAAAGTCTGTGACTAACCTGACCAAAACCGTCAATGAAACAAACAAACGTCGATGCCAAAGTAAAGCT
 GCAGAATCTGAAATAGAAAAGTTAAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCAGATACTGATGCCGCTCTG
 35 GATGCAACCACCAACGCCCTTGAATAAATTGGGAGAAAAATATAACGACATTTGCTGAAGAGACTAAGACAAATATCGTA
 AAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCCGAAGCATTAACGATATCGCCGATTC
 TTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCAATGAAGCCAAACAGACGGCGCAAGAAACCA
 CAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAGCCGAAGCTGCCGCTGGCACAGCTAATACT
 40 GCAGCCGACAAAGGCCGAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCTACGAACAAAGATAATATT
 GCTAAAAAAGCAACAGTGCAGGACGCTGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATACGATACTCGCTGAACGCT
 TTGGATAAAACAGTGTGAGACCTGCGCAAGAAACCGCCAAGGCTTGCAGAACAGCCGCGCTCTCCGGTCTGTTTC
 CAACCTTACAACGTGGGTCTCGAGCACCACCACCACCACCTGA

1 MTSAPDFNAG GTGIGSNSRA TTKSAAVSY AGIKNEMCKD RSMLCAGRDD
 45 51 VAVTDRDAKI NAPPNNLHTG DFPNPNDAYK NLINLKPAIE AGYTGRGVEV
 101 GIVDTGESVG SISFPELYGR KEHGYNENYK NYTAYMRKEA PEDGGGKDIE
 151 ASFDDEAVIE TEAKPTDIRH VKEIGHIDL V SHIIGGRSVD GRPAGGIAPD
 201 ATLHIMTND ETKNEMMVA IRNAWVKLGE RGVRIVNSF GTTSRAGTAD
 251 LFQIANSEEQ YRQALLDYS GDKTDEGIRL MQQSDYGNLS YHIRNKNMLF
 50 301 IFSTGNDAQA QPNTYALLPF YEKDAQKGII TVAGVDRSGE KFKREMYGEP
 351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG
 401 TAALLLQKYP WMSNDNLRTT LLTTAQDIGA VGVD SKFGWG LLDAGKAMNG
 451 PASFFFGDFT ADTKGTS DIA YSFRNDISGT GGLIKKGGSQ LQLHGNNTYT
 55 501 GKTIIEGGS L VLYGNKSDM RVETKGALIY NGAASGSLN SDGIVYLADT
 551 DQSGANETVH IKGSLQLDGK GTLYTRLGKL LKVDGTAIIG GKLYMSARGK
 601 GAGYLNSTGR RVFFLSAAKI GQDYSFFTNI ETDGGLLASL DSVEKTAGSE
 651 GDPLSYVRR GNAARTASAA AHSAPAGLKH AVEQGGSNLE NLMVELDASE
 701 SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANA DGVRIWFNSLA
 751 ATVYADSTAA HADMQRRRLK AVSDGLDHNG TGLRVIQATQ QDGGTWEQGG
 60 801 VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDSISLF
 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMQLGAL
 901 GGVNVFFAAT GDLTVEGGLR YDLLKQDAFA EKGSLGWSG NSLTEGTLVG
 951 LAGLKLSQLP SDKAVLFATA GVERDLNGRD YTVTGGFTGA TAATGKTGAR
 1001 NMPHTRLVAG LGADVEFGNG WNGLARYSYA GSKQYGNHSG RVGVGYRFLF
 65 1051 GGGGTGSATN DDDVKKAATV AIAAAYNNGQ EINGFKAGET IYDIDEDGTI
 1101 TTKDATAADV EADDFKGLGL KRVVTNLTKT VNEKNQNVDA KVKAASEIE
 1151 KLTTKLADTD AALADTDAAL DATTNALNKL GENITTFEE TKTNIKIDE

1201 KLEAVADTVD KHAEAFNDIA DSI DETNTKA DEAVKTANEA KQTAEETKQN
 1251 VDAKVKAET AAGKAEAAAG TANTAADKAE AVAAKVTDIK ADIATNKDNI
 1301 AKKANSADVY TREESDSKFV RIDGLNATTE KLDTRLASAE KSIADHDTRL
 1351 NGLDKTVSDL RKETRQGLAE QAALSGLFQP YNVGLEHHHH HH*

5

Example 4 – hybrids of $\Delta G741$

Protein 741 has the following sequence:

1 VNRTAFCCLS LTTALILTAC SSGGGGVAAD IGAGLADALT APLDHKDKGL
 51 QSLTLDQSVR KNEKLKLAQ GAERTYGNND SLNTGKLKND KVSRLFDFIRQ
 101 IEVDGQLITL ESSEFQVYKQ SHSALTAFQT EQIQDSEHSG KMAKRFQRI
 151 GDIAGEHTSF DKLPEGGGRAT YRGTAFGSDD AGGKLTYYTID FAAKQNGKI
 201 EHLKSPELNV DLAAADIKPD GKRHAVISGS VLYNQAEKGS YSLGIFGGKA
 251 QEVAGSAEVK TVNGIRHIGL AAKQ*

10

15

 $\Delta G741$ thus has the following basic sequence:

VAAD IGAGLADALT APLDHKDKGL
 QSLTLDQSVR KNEKLKLAQ GAERTYGNND SLNTGKLKND KVSRLFDFIRQ
 IEVDGQLITL ESSEFQVYKQ SHSALTAFQT EQIQDSEHSG KMAKRFQRI
 GDIAGEHTSF DKLPEGGGRAT YRGTAFGSDD AGGKLTYYTID FAAKQNGKI
 EHLKSPELNV DLAAADIKPD GKRHAVISGS VLYNQAEKGS YSLGIFGGKA
 QEVAGSAEVK TVNGIRHIGL AAKQ*

20

 $\Delta G741$ was fused directly in-frame upstream of proteins 961, 961c, 983 and ORF46.1:

25

 $\Delta G741-961$

ATGGTCGCCCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACCAGCACCCTCGACCATAAAGACAAAGGTTTGCAG
 TCTTTGACGCTGGATCAGTCCGTGAGAAAACGAGAACTGAACTGGCGGCACAAGGTGCGGAAAAAAGTTATGGA
 AACGGTGACAGCCTCAATACGGGCAAATTGAAGAACGACAAGGTGAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
 GACGGGCAGCTCATTACCTTGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTCAG
 30 ACCGAGCAATACAAAGATTCCGAGCATTCGGGAAGATGGTTGCGAAACGCCAGTTTCAAGTATCGCGGACGCGGCTTCGTTTACAGACGATGCC
 GAACATACATCTTTTGACAAGCTTCCCGAAGCGCGGCGGCGACATATCGCGGACGCGGCTTCGTTTACAGACGATGCC
 GGCGGAAAACTGACCTACACCATAGATTTCGCGGCCAAGCAGGGAAACGCCAAATCGAACATTTGAAATCGCCAGAA
 CTCAATGTCGACCTGGCCGCCGCGGATATCAAGCCGATGGAACGCCATGCGGTCATCAGCGGTTCCGTCTCTTTAC
 AACCAAGCCGAGAAAGGCAGTTACTCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTG
 35 AAAACCGTAAACGGCATACGCCATATCGGCCTTGCCGCCAAGCAACTCGAGGGTGGCGGAGGCACTGGATCCGCCACA
 AACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCTTACAAACAAATGGCCAAAGAAATCAACGGTTTC
 AAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAA
 GCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCAGCAAAACCGTCAATGAAAAACAAACAAAC
 40 GTCGATGCCAAAGTAAAGCTGCAGAACTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTA
 GCAGATACTGATGCCGCTCTGGATGCAACCAACGCTTGAATAAATTTGGGAGAAAATATAACGACATTTGCTGAA
 GAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCA
 TTCAACGATATCGCCGATTCAATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCCAATGAAGCCAAA
 CAGACGGCCGAGAAACCAAAACAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAGCCGAAGCT
 45 GCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAAAGCTGATATC
 GCTACGAACAAAGATAATATTGCTAAAAAGCAACAGTGCCGACGTGTACACAGAGAAGAGTCTGACAGCAAAATTT
 GTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGAT
 CACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACAGCTGCGCAAAGAACCCGCAAGGCCTTGAGAACAA
 GCCCGCTCTCCGGTCTGTTCAACCTTACAACGTGGGTTCGGTTCAATGTAACCGCTGCAAGTTCGGCGGCTACAAATCC
 50 GAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACTTTGCCGCCAAAGCAGGCGTGGCAGTCCGCAC
 TCGTCCGCTTCTTCCGACGCTACCATGTCGGCGTCAATTACGAGTGGCTCGAGCACCACCACCACCACCTGA

50

1 MVAADIGAGL ADALTAFLDH KDKGLQSLFL DQSVRKNEKL KLAAQGAERT
 51 YNGDSLNTG KLKNDKVSF DFIRQIEVDG QLITLESSEF QVYKQSHSAL
 101 TAFQTEQIQD SEHSGKMAK RFIRIGDIAG EHTSFDKLPE GGRATYRGTA
 151 FGSDDAGGKL TYTIDFAAQ GNGKIEHLKS PELNVDLAAA DIKPDGKRHA
 201 VISGSVLYNQ AEKGSYSLGI FGGKAQEVAG SAEVKTVNGI RHIGLAAKQL
 251 EGGGTGSAT NDDDVKKAAT VAIAAAYNNG QEINGFKAGE TIYDIDEDGT
 301 ITRKDATAAD VEADDFKGLG LKKVVTNLTK TVNENKQNV AKVKAASEI
 351 EKLTTKLADT DAALADTDA LDATTNALNK LGENITTFAE ETKTNIVKID
 60 401 EKLEAVADTV DKHAEAFNDI ADSLDETNTK ADEAVKTANE AKQTAEETKQ

55

60

-20-

451 NVDAKVKAAE TAAGKAEAAA GTANTAADKA EAVA AKVTDI KADIATNKDN
501 IAKKANSADV YTREESDSKF VRIDGLNATT EKLDTRLASA EKSIADHDTR
551 LNGLDKTVSD LRKETRQGLA EQAALSGLFQ PYNVGRFNV TAAVGGYKSES
601 AVAIGTGFRF TENFAAKAGV AVGTSSGSSA AYHVG VNYEW LEHHHHHHH*

5

AG741-961c

ATGGTCGCCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACC GCACCGCTCGACCATAAAGACAAAGGTTTGCAG
TCTTTGACGCTGGATCAGTCCGT CAGGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGA
AACGGTGACAGCCTCAATACGGGCAAATTGAAGAACGACAAGGT CAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
GACGGGCAGCTCATTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTTCAG
ACCGAGCAAATACAAGATTCCGAGCATTCCGGGAAGATGGTTGCGAAACGCCAGTT CAGAATCGCGGACATAGCGGGC
GAACATACATCTTTTGACAAGCTTCCCGAAGCGGCAGGGCGACATATCGCGGGACGGCGTTCCGTT CAGACGATGCC
GGCGGAAAACTGACCTACACCATAGATTTCGCCCGCCAAGCGGAAACGCAAAATCGAACATTTGAAATCGCCAGAA
CTCAATGTCGACCTGGCCGCCGCCGATATCAAGCCGATGGAAAAACGCCATGCCGTCATCAGCGTTCCGTCCTTTAC
AACCAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGCGCAGCGCGGAAGTG
AAAACCGTAAACGCATACGCCATATCGGCCTTGCCGCCAAGCAACTCGAGGGTGCGCGAGGCAGCTGGATCCGCCACA
AACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCTTACAACAATGGCCAAGAAATCAACGGTTTC
AAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAA
GCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGTGACTTAACCTGACCAAAAACCTCAATGAAAAACAAACAAAAC
GTCGATGCCAAAGTAAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTA
GCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTTGAATAAATGGGAGAAAATATAACGACATTTGCTGAA
GAGACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGCTGATACCTGCGACAAGCATGCCGAGCA
TTCAACGATATCGCCGATTCAATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCAATGAAGCCAAA
CAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAGCCGAAGCT
GCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATC
GCTACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGACAGCAAATTT
GTCAGAAATTGATGCTGTAACGCTACTACCGAAAAATTGGACACACGCTTGCTTCTGCTGAAAAATCCATTGCCGAT
CACGATACTCGCCGTAACGGTTTGATGATAAACAGTGTGACACCTGCGCAAGAAACCCGCCAAGGCTTGCAGAACAA
GCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTCTCGAGCACCACCACCACCACCTGA

1 MVAADIGAGL ADALTAPLDH KDKGLQSLTL DQSVRKNEKL KLAAQGAETK
51 YNGDSLNTG KLKNDKVS RF DFIRQIEVDG QLITLESGEF QVYKQSHSAL
101 TAFQTEQIQD SEHSGKMVAK RQFRIGDIAG EHTSFDKLPE GGRATYRGTA
151 FGSDDAGGKL TYTIDFAAKQ GNGKIEHLKS PELNVDLAAA DIKPDGKRHA
201 VISGSVLYNQ AEKGSYSLGI FGGKAQEVAG SAEVKT VNGI RHIGLAAKQL
251 EGGGTGSAT NDDDVKKAAT VAIAAAYNNG QEINGFKAGE TIYDIDEDGT
301 ITKKDATAAD VEADDFKGLG LKKVVTNLTK TVNENKQND AKVKAASEI
351 EKLTTKLADT DAALADTDAE LDATTNALNK LGENITTFAE ETKTNI VKID
401 EKLEAVADTV DKHAEAFNDI ADSLDETNTK ADEAVKTANE AKQTAETKQ
451 NVDAKVKAAE TAAGKAEAAA GTANTAADKA EAVA AKVTDI KADIATNKDN
501 IAKKANSADV YTREESDSKF VRIDGLNATT EKLDTRLASA EKSIADHDTR
551 LNGLDKTVSD LRKETRQGLA EQAALSGLFQ PYNVGL EHHH HHH*

45

AG741-983

ATGGTCGCCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACC GCACCGCTCGACCATAAAGACAAAGGTTTGCAG
TCTTTGACGCTGGATCAGTCCGT CAGGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGA
AACGGTGACAGCCTCAATACGGGCAAATTGAAGAACGACAAGGT CAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
GACGGGCAGCTCATTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTTCAG
ACCGAGCAAATACAAGATTCCGAGCATTCCGGGAAGATGGTTGCGAAACGCCAGTT CAGAATCGCGGACATAGCGGGC
GAACATACATCTTTTGACAAGCTTCCCGAAGCGGCAGGGCGACATATCGCGGGACGGCGTTCCGTT CAGACGATGCC
GGCGGAAAACTGACCTACACCATAGATTTCGCCCGCCAAGCAGGGAACGCGCAAAATCGAACATTTGAAATCGCCAGAA
CTCAATGTCGACCTGGCCGCCGCCGATATCAAGCCGATGGAAAAACGCCATGCCGTCATCAGCGGTTCCGTCCTTTAC
AACCAAGCTCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGCGCAGCGCGGAAGTG
AAAACCGTAAACGCATACGCCATATCGGCCTTGCCGCCAAGCAACTCGAGGGATCCGGCGAGGCGGCACTTCTGCG
CCCGACTTCAATGACGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCAGCAGTATCTTAC
GCCGATATCAAGAACGAAATGTCAAAGACAGAAGCATGCTCTGTGCCGGTTCGGGATGACGTTGCCGTTACAGACAGG
GATGCCAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCATACAAGAAATTTG
ATCAACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACCGGGGTAGAGGTAGGTATCGTGCACACAGGCGAATCC
GTCGCGCAGCATCTCTTTCCGGAATGTATGGCAGAAAAGAACCGGCTATAACGAAAATACAAAACTATACGGCG
TATATGCGGAAGGAAGCGCCTGAAGACGGAGCGGTAAAGACATTGAAGCTTCTTTCCAGCATGAGGCGCTTATAGAG
ACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTTGGTCTCCCATATTATTGGCGGG
CGTTCCGTTGACGGCAGACCTGCAGGCGGTATGCGCCCGATGCGACGCTACACATAATGAATACGAATGATGAAACC
AAGAACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGCGCAACGTGGCGTGCAGCATCGTCAATAAC
AGTTTGTGAACACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAGTACCGCCAAGCG
TTGCTCGACTATTCCGGCGGTGATAAAACAGACGAGGGTATCCGCTGATGCAACAGAGCGATTACGGCAACCTGTCC

65

TACCACATCCGTAATAAAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACATATGCC
 CTATTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGCGAGCGTAGACCGCAGTGAGAAAAAGTTC
 AAACGGGAAATGTATGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTTGCGGAATTACTGCCATGTGG
 5 TGCTGTGTCGGCACCCCTATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTTGCCGGAACATCCTTTTCC
 GCACCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGTACCACG
 TTGCTGACGACGGCTCAGGACATCCGGTGCAGTCCGGCTGGACAGCAAGTTCGGCTGGGAGCTGCTGGATGCGGGTAAG
 GCCATGAACGGACCCCGCTCTTTCCGTTCCGGCGACTTTACCGCGGATACGAAAGGTACATCCGATATTGCCTACTCC
 TTCCGTAACGACATTTACGGCACGGCGGCCTGATCAAAAAGGCGGCAGCCAACCTGCAACTGCACGGCAACAACACC
 10 TATACGGGCAAAACCATTTATCGAAGGCGGTTCCGTTGGTGTGTACGGCAACAACAAATCGGATATGCGCGTCGAAACC
 AAAGGTGCGCTGATTATATAACGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTTGTCTATCTGGCAGATACC
 GACCAATCCGGCGCAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAGGTACGCTGTACACACGT
 TTGGGCAAACTGCTGAAAGTGGACGGTACGGCGATTATTCGGCGGCAAGCTGTACATGTTCGGCAGCGGCAAGGGGCA
 GGCTATCTCAACAGTACCGGACGAGCTGTTCCCTTCTGAGTCCGCGCAAAATCGGGCAGGATTATTTCTTTTCACACA
 15 AACATCGAAACCGACGGCGGCCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAAGGCGACACGCTG
 TCCTATTATGTCCGTCGCGGCAATGCGGCACGAGTGTCTTCGGCAGCGGCACATTCGCGCGCCCGCGGTCTGAAACAC
 GCCGTAGAACAGGGCGGCAGCAATCTGGAACCTGATGGTCGAACTGGATGCCTCCGAAATCATCCGCAACACCCGAG
 ACGGTTGAAACTGCGGCAGCCGACCGCACAGATATGCCGGCATCCGCCCTACGGCGCAACTTTCCGCGCAGCGGCA
 20 GCGGTACAGCATGCGAATGCCCGCGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGCTATGCTCCGACAGTACC
 GCGCGCATGCCGATATGCAGGGACGCGCCTGAAAGCCGTATCGGACGGGTGGACCAACCGGCACGGGCTTCGCGC
 GTCATCGCGCAAAACCAACAGGACGGTGGAACTGGGAACAGGGCGGTGTTGAAGGCAAAATGCGCGGCAGTACCCAA
 ACCGTTCGGCATTTGCCGCAAAACCGCGCAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGACGACATGGAGC
 GAAACAGTGCAAATGCAAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCACGATGCGGGCGATATCGGCTAT
 25 CTCAAAGGCTGTTCTCCTACGGACGCTACAAAACAGCATACGCCGACGACCCGTTGCGGACGAAACATGCGGAAGGC
 AGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTTCGCGCAACGGGAGATTGTAGC
 GTCGAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTTCGCGCAAAAAGGCAGTGTCTTGGGCTGGAGCGGC
 AACAGCCTCACTGAAGGCACGCTGGTTCGACTCGCGGTCTGAAGCTGTGCAACCCCTTGAGCGATAAAGCCGTCCTG
 TTTGCAACGGCGGGCGTGAACGCGACCTGAACGCGACGCTACACGGTAACGGGCGGCTTTACCGCGCGGACTGCA
 30 GCAACCGGCAAGACGGGGGACGCAATATGCCGACACCCGCTGTTGTCGGGCTGGGCGGGATGTCGAATTCGGC
 AACGGCTGGAACGGCTTGGCACGTTACAGCTACGCCGTTTCCAAACAGTACGGCAACCACAGCGGACGAGTCCGCGTA
 GGCTACCGGTTCTCTGAGCACCACCACCACCACCTGA

1 MVAADIGAGL ADALTAPLDH KDKGLQSLTL DQSVRKNEKL KLAAQGAETK
 51 YGNGLSLNTG KLKNDKVSFR DFIRQIEVDG QLITLESGEF QVYKQSHSAL
 101 TAFQTEQIQD SEHSGKMVAK RQFRIGDIAG EHTSFDKLE GGRATYRGTA
 35 151 VSGDDAGGKL TYTIDFAAQK GNGKIEHLKS PELNVDLAAA DIKPDGKRHA
 201 FGSISVLYNQ AEKGSYSLGI FGGKAQEVAG SAEVKTVNGI RHIGLAAKQL
 251 ESGSGGGTSA PDFNAGGTGI GSNSRATTAK SAAVSYAGIK NEMCKDRSML
 301 CAGRDDVAVT DRDAKINAPP PNLHTGDFPN PNDAYKNLIN LKPAIEAGYT
 351 GRGVEVGIVD TGESVGSISF PELYGRKEHG YNENYKNYTA YMRKEAPEDG
 40 401 GGKDIEASFD DEAVIETEA PTDIRHVKEI GHIDLVSII GGRSVDGRPA
 451 GGIAPDATH IMNTNDETKN EMMVAAIRNA WVKLGERGVR IVNNSFGTTS
 501 RAGTADLFQI ANSEEQYRQA LLDYSGGDKT DEGIRLMQOS DYGNLSYHIR
 551 NKNMLFIFST GNDAQAQPN YALLPFYEK AOKGIITVAG VDRSGEKFPR
 601 EMYGEPGTEP LEYGSNHCGI TAMWCLSAFY EASVRFRTRN PIQIAGTSFS
 45 651 APIVTGTAAL LLQKYPWMSN DNLRTLLLT AQDIGAVGVD SKFGWGLLDA
 701 GKAMNGPASF PFGDPTADTK GTSDIAYSFR NDISGTGLI KKGSQQLHL
 751 GNNTYTGTI IEGGSLVLYG NNKSDMRVET KGALIYNGAA SGGSLNSDGI
 801 VYLADTDQSG ANETVHIKGS LQLDGKGLY TRLGKLLKVD GTAIIGKLY
 851 MSARGKGAGY LNSTGRRVPF LSAKIGQDY SFFTNIETDG GLLASLDSVE
 50 901 KTAGSEGD TL SYVVRGNAA RTASAAHSA PAGLKHAVEQ GGSNLENLMV
 951 ELDASESSAT PETVETAAAD RTDMPGIRPY GATFRAAAV QHANAADGVR
 1001 IFNSLAATVY ADSTAAHADM QGRRLLKAVSD GLDHNGTGLR VIAQTQDDGG
 1051 TWEQGGVEGK MRGSTQTVGI AAKTGENTTA AATLGMGRST WSENSANAKT
 1101 DSISLFAGIR HDAGDIGYK GLFSYGRYKN SISRSTGADE HAEGSVNGTL
 55 1151 MQLGALGGVN VPFAATGDLT VEGGLRYDLL KQDAFAEKG ALGWSGNSLT
 1201 BGTLVGLAGL KLSQPLSDKA VLFATAGVER DLNGRDYTVT GGFTGATAAT
 1251 GKTGARNMPH TRLVAGLGAD VEFNGWNGL ARYSYAGSKQ YGNHSGRVGV
 1301 GYRFLEHHHH HH*

60

AG741-ORF46.1

ATGGTTCGCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACCGCACCGCTCGACCATAAAGACAAAGGTTTCAG
 TCTTTGACGCTGGATCAGTCCGTACAGAAAAACGAGAACTGAGCTGGCGGCACAAGGTGCGGAAAAAATTTATGGA
 AACGGTGACAGCCTCAATACGGGCAAAATGAAGAACGACAAGGTCAGCCGTTTCGACTTTATCCGCCAAATCGAAGTG
 65 GACGGGCGAGCTCATTAACCTTGGAGAGTGGAGATTCAAGTATACAAACAAAGCCATTCCGCTTAACCGCTTTTCAG
 ACCGAGCAAAATACAAGATTTCGAGCATTCGGGGAAGATTGGTTCGAAACGCCAGTTTCAGAAATCGGCGACATAGCGGC
 GAACATACATCTTTTGACAAGCTTCCCGAAGGCGGCAGGGCGACATATCGCGGACGGGCTTCGGTTACAGCATGCC

5 GCGGAAAACTGACCTACACCATAGATTTGCGCCCAAGCAGGAAACGGCAAAATCGAACATTTGAAATCGCCAGAA
 CTCAATGTCGACCTGGCCGCCGCGATATCAAGCCGGATGGAACCGCCATGCCGTCATCAGCGGTTCCGTCCTTTAC
 AACCAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTG
 AAAACCGTAAACGGCATAACGCATATCGGCCTTGCCGCCAAGCAACTCGACGGTGGCGGAGGCACTGGATCCTCAGAT
 10 TTGGCAAACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTTGAACCCGACGGGAAATACCACCTATTCCGGC
 AGCAGGGGGGAACTTGCCGAGCGCAGCGGCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTGATG
 ATTCAACAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCACGGGCACGAAGTCCATTCCCCC
 TTCGACAACCATGCCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGAATTTAGCCTTTACCGCATCCAT
 TGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGCGGCTATCCCGCTCCCAAAGGCGCG
 15 AGGGATATATACAGCTACGACATAAAAGGCGTTGCCCAAATATCCGCCTCAACCTGACCGACAACCGCAGCACCGBA
 CAACGGCTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAACCGGCCACC
 CGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTTAAAAACATC
 ATCGGCGCGCAGGAGAAATGTGCGGCGCAGCGCATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATTGCTGTCTATG
 CACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTGGCAGATATGGCGCAACTCAAAGAC
 TATGCCGAGCAGCCATCCGCGATTGGGCAGTCCAAAACCCCAATGCCGCGACAAGGCATAGAAGCCGTCAGCAATATC
 20 TTTATGGCAGCCATCCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCACGGCACATCCT
 ATCAAGCGGTGCGAGATGGGCGCGATCGCATTTCCGAAAGGGAATCCGCCGTCAGCGACAATTTTGGCGATGCGGCA
 TACGCCAAATACCCGTCCTTACCATTTCCGAAATATCCGTTCAAACCTGGAGCAGCGTTACGGCAAAGAAAACATC
 ACCTCCTCAACCGTGCCGCCGTCAAACGGCAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTACCG
 TTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCACGTGAAATATGATACGCTCGAGCACCACCACCACCACCTGA

1 MVAADIGAGL ADALTAPLDH KDKGLQSLTL DQSVRKNEKL KLAAQGAETK
 51 YNGDSLNTG KLKNDKVSFR DFIRQIEVDG QLITLESGEF QVYKQSHSAL
 101 TAFQTEQIQD SEHSGKMVAK RQFRIGDIAG EHTSFDKLPE GGRATYRGTA
 25 151 FGSDDAGGKL TYTIDFAAQ GNGKIEHLKS PELNVDLAAA DIKPDGKRHA
 201 VISGSVLYNQ AEKGSYSLGI FGGKAQEVAG SAEVKTVNGI RHIGLAAKQL
 251 DGGGGTGSSD LANDSFIRQV LDRQHFPEDG KYHLFGSRGE LAERSGHIGL
 301 GKIQSHQLGN LMIQQAAIKG NIGYIVRFS D HGHEVHSPFD NHASHSDSDE
 351 AGSPVDGFSL YRIHWDGYEH HPADGYDGPQ GGGYPAPKGA RDIYSYDIKG
 30 401 VAQNIRLNL T DNRSTGQRLA DRFHNA G SML TQGVGDGFKR ATRYSPELDR
 451 SGNAAEAFNG TADIVKNIIG AAGEIVGAGD AVQGISSEGSN IAVMHGLGLL
 501 STENKMARIN DLADMAQLKD YAAAAIRDWA VQNPNAAQGI EAVSNIFMAA
 551 IPIKGIGAVR GKYGLGGITA HPIKRSQMG A IALPKGKSAV SDNFADAAYA
 601 KYPSPYHSRN IRSNLEQRYG KENITSS T V P PSNGKNVCLA DQRHPKTGVP
 35 651 FDGKGFPNFE KHVKYDTLEH HHHHH*

Example 5 – hybrids of 287

Expression of 287 as full-length with a C-terminal His-tag, or without its leader peptide but
 with a C-terminal His-tag, gives fairly low expression levels. Better expression is achieved
 40 using a N-terminal GST-fusion. As an alternative to using GST as an N-terminal fusion
 partner, 287 was placed at the C-terminus of protein 919 ('919-287'), of protein 953 ('953-
 287'), and of proteins ORF46.1 ('ORF46.1-287'). In both cases, the leader peptides were
 deleted, and the hybrids were direct in-frame fusions.

To generate the 953-287 hybrid, the leader peptides of the two proteins were omitted by
 45 designing the forward primer downstream from the leader of each sequence; the stop codon
 sequence was omitted in the 953 reverse primer but included in the 287 reverse primer. For
 the 953 gene, the 5' and the 3' primers used for amplification included a *NdeI* and a *BamHI*
 restriction sites respectively, whereas for the amplification of the 287 gene the 5' and the 3'
 primers included a *BamHI* and a *XhoI* restriction sites respectively. In this way a sequential
 50 directional cloning of the two genes in pET21b+, using *NdeI-BamHI* (to clone the first gene)
 and subsequently *BamHI-XhoI* (to clone the second gene) could be achieved.

The 919-287 hybrid was obtained by cloning the sequence coding for the mature portion of 287 into the *Xho*I site at the 3'-end of the 919-His clone in pET21b+. The primers used for amplification of the 287 gene were designed for introducing a *Sal*I restriction site at the 5'- and a *Xho*I site at the 3'- of the PCR fragment. Since the cohesive ends produced by the *Sal*I and *Xho*I restriction enzymes are compatible, the 287 PCR product digested with *Sal*I-*Xho*I could be inserted in the pET21b-919 clone cleaved with *Xho*I.

The ORF46.1-287 hybrid was obtained similarly.

The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens:

	Mixture with 287	Hybrid with 287
919	32000	16000
953	8192	8192
ORF46.1	128	8192

Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained for 919-287 and 953-287:

	919		953		ORF46.1	
<i>Strain</i>	<i>Mixture</i>	<i>Hybrid</i>	<i>Mixture</i>	<i>Hybrid</i>	<i>Mixture</i>	<i>Hybrid</i>
MC58	512	1024	512	1024	-	1024
NGH38	1024	2048	2048	4096	-	4096
BZ232	512	128	1024	16	-	-
MenA (F6124)	512	2048	2048	32	-	1024
MenC (C11)	>2048	n.d.	>2048	n.d.	-	n.d.
MenC (BZ133)	>4096	>8192	>4096	<16	-	2048

Hybrids of ORF46.1 and 919 were also constructed. Best results (four-fold higher titre) were achieved with 919 at the N-terminus.

Hybrids 919-519His, ORF97-225His and 225-ORF97His were also tested. These gave moderate ELISA titres and bactericidal antibody responses.

As hybrids of two proteins A & B may be either NH₂-A-B-COOH or NH₂-B-A-COOH, the "reverse" hybrids with 287 at the N-terminus were also made, but using ΔG287. A panel of strains was used, including homologous strain 2996. FCA was used as adjuvant:

	287 & 919		287 & 953		287 & ORF46.1	
Strain	$\Delta G287-919$	919-287	$\Delta G287-953$	953-287	$\Delta G287-46.1$	46.1-287
2996	128000	16000	65536	8192	16384	8192
BZ232	256	128	128	<4	<4	<4
1000	2048	<4	<4	<4	<4	<4
MC58	8192	1024	16384	1024	512	128
NGH38	32000	2048	>2048	4096	16384	4096
394/98	4096	32	256	128	128	16
MenA (F6124)	32000	2048	>2048	32	8192	1024
MenC (BZ133)	64000	>8192	>8192	<16	8192	2048

Better bactericidal titres are generally seen with 287 at the N-terminus.

When fused to protein 961 [NH₂- $\Delta G287-961$ -COOH – sequence shown above], the resulting protein is insoluble and must be denatured and renatured for purification. Following renaturation, around 50% of the protein was found to remain insoluble. The soluble and insoluble proteins were compared, and much better bactericidal titres were obtained with the soluble protein (FCA as adjuvant):

	2996	BZ232	MC58	NGH38	F6124	BZ133
Soluble	65536	128	4096	>2048	>2048	4096
Insoluble	8192	<4	<4	16	n.d.	n.d.

Titres with the insoluble form were, however, improved by using alum adjuvant instead:

Insoluble	32768	128	4096	>2048	>2048	2048
------------------	-------	-----	------	-------	-------	------

- 961c was also used in hybrid proteins (see above). As 961 and its domain variants direct efficient expression, they are ideally suited as the N-terminal portion of a hybrid protein.

Example 23 – further hybrids

Further hybrid proteins of the invention are shown in the drawings and have the sequences set out below. These are advantageous when compared to the individual proteins:

15

ORF46.1-741

20

ATGTCAGATTTGGCAAACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTTGAACCCGACGGGAAATACCAC
 CTATTCGGCAGCAGGGGGAACTTGCCGAGCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGC
 AACCTGATGATTCAACAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCACGGGCACGAAGTC
 CATTCCTCCCTTCGACAACCATGCTTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTTAC
 CGCATCCATTGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGCGCTATCCCGCTCCC
 AAAGGCGGAGGGATATATACAGCTACGACATAAAAGGCGTTGCCAAAATATCCGCCTCAACCTGACCGACAACCGC
 AGCACCGACAACGGCTTGCCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAA
 CGCGCCACCCGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCAAGCCTTCAACGGCACTGCAGATATCGTT
 AAAACATCATCGGCGCGGCAGGAGAAATTGTCCGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATT

5 GCTGTCATGCACGGCTTGGGTCTGCTTTCCACCGAAAAACAAGATGGCGCGCATCAACGATTGGCAGATATGGCGCAA
 CTCAAAGACTATGCCGAGCAGCCATCCCGGATTGGGCGAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTC
 AGCAATATCTTTATGGCAGCCATCCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCAGC
 GCACATCCTATCAAGCGGTGCGAGATGGGCGCGATCGCATTGCCGAAAGGGAAATCCGCCGTCAGCGACAATTTTGCC
 10 GATGCGGCATACGCCAAATACCCGTCCTTACCATTCCCGAAATATCCGTTCAAACCTTGGAGCAGCGTTACGGCAA
 GAAAACATCACCTCCTCAACCGTGCCGCGTCAAACGGCAAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACA
 GCGGTACCGTTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCACGTGAAATATGATACGGGATCCGGAGGGGGTGGT
 GTCGCGCGCGACATCGGTGCGGGGCTTGCCGATGCACTAACCGCACCGCTCGACCATAAAGACAAAGGTTTGACGTCT
 TTAGCGCTGGATCAGTCCGTGAGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGAAC
 15 GGTGACAGCCTCAATACGGGCAAATTGAAGAACGACAAGGTGAGCCGTTTCGACTTTATCCGCCAAATCGAAGTGGAC
 GGGCAGCTCATTACCTTGGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCCTTAACCGCCTTTCAGACC
 GAGCAAAATACAGATTTCGGAGCATTCGGGAAGATGGTTGCGAAACGCCAGTTCAGAACTCGGCGACATAGCGGGCGAA
 CATACATCTTTTGACAAGCTTCCCGAAGCGCGGAGGCGACATATCGCGGGACGGCGTTCGCTTCAGACGATGCGCGG
 GGAAAACTGACCTACACCATAGATTTGCGCGCAAGCAGGGAACCGGCAAAATCGAACATTTGAAATCGCCAGAACTC
 20 AATGTCGACCTGGCCGCGCGCATATCAAGCCGATGGAACGCCATGCGCTCATCAGCGGTTCCGTCTTTACAAC
 CAAGCCGAGAAAGGCAGTTACTCCCTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGCGAGCGCGGAAGTGAAA
 ACCGTAAACGGCATACGCCATATCGGCTTGCCGCCAAGCAACTCGAGCACCACCACCACCACCTGA

20 1 MSDLANDSFI RQVLDROHFE PDGKYHLFGS RGE LAERSGH IGLGKIQSHQ
 51 LGNLMIQQA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
 101 FSLYRIHWDG YEHHPADGYD GPQGGGYAP KGARDIYSYD IKGVAQNIRL
 151 NLTDNRSTGQ RLADRFHNAG SMLTQGVGDG FKRA TRYSP E LDRSGNAABEA
 201 FNGTADIVKN IIGAAGEIVG AGDAVQGISE GSNIAVMHGL GLLSTENKMA
 251 RINDLADMAQ LKDYAAAAIR DWAVQNPNA QGIEAVSNIF MAAPIKIGI
 25 301 AVRKYGLGG ITAHPKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
 351 SRNIRSNLEQ RYKKNITSS TVPPSNGKNV KLADQRHPKT GVPFDGKGFP
 401 NFEKHVKYDT GSGGGVAAAD IGAGLADALT APLDHKDKGL QSLTLDQSVR
 451 KNEKLKLAQ GAEKTYGNGD SLNTGKLKND KVS RFD FIRQ IEVDGQLITL
 501 ESGEFQVYKQ SHSALTAFQT EQIQDSEHS KMVAKRQFRI GDIAGEHTSF
 30 551 DKLPEGGRAT YRGTAFGSDD AGGKLTYTID FAKQNGKI EHLKSPELNV
 601 DLAAADIKPD GKRHAIVSGS VLYNQAEKGS YSLGIFGGA QEVAGSAEVK
 651 TVNGIRHIGL AAKQLEHHHH HH*

35 ORF46.1-961

ATGTCAGATTGGCAAACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTGCAACCCGACGGGAAATACCAC
 CTATTCGGCAGCAGGGGGAACTTGCCGAGCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGC
 AACCTGATGATTCAACAGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCAGGGCAGGAAGTC
 40 CATCCCCCTTCGACAACCATGCCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTTAC
 CGCATCCATTGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGCGGCTATCCCGCTCCC
 AAAGGCGCGAGGGATATATACAGCTACGACATAAAAGCGCTTGCCCAAATATCCGCCTCAACCTGACCGACAACCGC
 AGCACCGGACACGGCTTGCCGAGCGTTCCCAATGCCGTTAGTAGCTGACGCAAGTAGGCGCAGGATTCAAA
 CGCGCCACCCGATACAGCCCCGAGCTGGACAGATCGGGCAATGCCGCCAAGCCTTCAACCGCACTGACAGATATCGTT
 45 AAAAAATCATCGGCGCGGAGGAGAAATGTGCGCGCAGGCGATGCCGTGACGGGCATAAGCGAAGGCTCAAACATT
 GCTGTCATGCACGGCTTGGGTCTGCTTTCCACCGAAAAACAAGATGGCGCGCATCAACGATTGGCAGATATGGCGCAA
 CTCAAAGACTATGCCGAGCAGCCATCCCGGATTGGGCGAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTC
 AGCAATATCTTTATGGCAGCCATCCCCATCAAAGGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCAGC
 GCACATCTATCAAGCGGTGCGAGATGGGCGCGATCGCATTCGCGGAAAGGAAATCCGCCGACGACATTTTGCC
 50 GATGCGGCATACGCCAAATACCCGTCCTTACCATTCCCGAAATATCCGTTCAAACCTTGGAGCAGCGTTACGGCAA
 GAAAACATCACCTCCTCAACCGTGCCGCGTCAAACGGCAAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACA
 GCGGTACCGTTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCACGTGAAATATGATACGGGATCCGGAGGAGGAGGA
 GCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAGAAATCAAC
 GTTTCAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAGACGCAACTGCAGCCGAT
 55 GTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAAACAAA
 CAAAACGTCGATGCCAAAGTAAAAGCTGCGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCC
 GCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAATATAACGACATTT
 GCTGAAGAGACTAAGACAAATATCGTAAAAATGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAGCATGCC
 GAAGCATTCAACGATATCGCGGATTCATTGGATGAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCAATGAA
 60 GCCAAACAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAAGCTGCAGAACTGCAGCAGGCAAGGCC
 GAAGCTGCGCTGGCACAGCTAATACCTGCAGCCGACAAAGGCTGTCGCTGCAAAAAGTTACCGACATCAAAGCT
 GATATCGCTACGAACAAAGATAATATTGCTAAAAAAGCAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGACAGC
 AAATTTGTGAGAATTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCCATT
 65 GCCGATCACGATACTCGCTGAACGGTTTGGATAAAACAGTGTCAGACCTGCGCAAAAGAAACCCGCCAAGGCCCTTGCA
 GAACAAGCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCGCGCGGTAC
 AAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAATTTGCGGCCAAAGCAGGCGTGGCAGTC
 GGCACCTTCGTCGGTTCTTCCGAGCCTACCATGTGCGCGTCAATTACGAGTGGCTCGAGCACCACCACCACCACCTGA

1 MSDLANDSFI RQVLDRQHFE PDGKYHLFGS RGLAERSGH IGLGKIQSHQ
51 LGNLMIQQAA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
5 FSLYRIHWDG YEHPADGYD GPQGGYPAP KGARDIYSYD IKGVAQNIRL
151 NLTDNRSTGQ RLADRFHNAG SMLTQGVGDG FKRATRYSP E LDRSGNAAEA
201 FNGTADIVKN IIGAAGEIVG AGDAVQGIS E GSNIAVMHGL GLLSTENKMA
251 RINDLADMAQ LKDYAAAAIR DWAVQNPNA QGIEAVSNIF MAAIPIKGIG
301 AVRKYGLGG ITAHPIKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
10 SRNIRSNLEQ RYKKNITSS TVPPSNGKNV KLADQRHPKT GVPFDGKGF
401 NFEKHVKYDT GSGGGGATND DDVKAATVA IAAAYNNGQE INGFKAGETI
451 YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTV NENKQNVDAK
501 VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKL E ENITTFAEET
551 KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNTKAD EAVKTANEAK
601 QTAETKQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA VAAKVTDIKA
15 DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK LDTRLASAEK
701 SIADHDTRLN GLDKTVSDLR KETRQGLAEQ AALSGLFPQY NVGRFNVTA
751 VGGYKSESAV AIGTGFRFTE NFAAKAGVAV GTSSGSSAAY HVGVNYEWLE
801 HHHHHH*

ORF46.1-961c

ATGTCAGATTTGGCAAACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTCGAACCCGACGGGAAATACCAC
CTATTCGGCAGCAGGGGGGAACCTGCGGAGCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGC
25 AACCTGATGATTCACAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCAGGGCAGCAAGTC
CATTTCCCTTCGACAACCATGCCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTTAC
CGCATCCATTGGGACGGATACGAACACCATCCCGCGCAGCGCTATGACGGGCCACAGGGCGGCGGTATCCCGTCCC
AAAGGCGCGAGGATATATACAGCTACGACATAAAAGCGCTTGCCCAAATATCCGCTCAACCTGACCGACAACCGC
30 AGCACCGGACAACGCTTGCCGACCGTTTCCACAATGCCGCTAGTATGCTGACGCAAGGATAGGCGACGGATTCAA
CGCGCCACCCGATACAGCCCGAGCTGGACAGATCGGGCAATGCCGCGAAGCCTTCAACGGCACTGCAGATATCGTT
AAAACATCATCGGCGCGGAGAGAAATGTGCGCGCAGGCGATGCCGTGACGGGCATAAGCGAAGGCTCAAACATT
GCTGTCTATGCACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTGTCAGATATGGCGCAA
CTCAAAGACTATGCCGACGAGCCATCCCGGATTGGGCAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTC
35 AGCAATATCTTTATGGCAGCCATCCCATCAAAGGGATTGGAGCTGTTGGGGAAAAATACGGCTTGGGCGGCATCAG
GCACATCTATCAAGCGGTCGAGATGGGCGCGATCGCATTCGCGAAAGGAAATCCGCGCTAGCGACAATTTTGGC
GATGCGGCATACGCCAAATACCCGTCCTTACCATTCCCGAAATATCCGTTCAAACCTGGAGCAGCGTTACGCCAAA
GAAACATCACCTCTCAACCGTGCCGCGCTCAAACGGCAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACA
GGCGTACCGTTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCACGTGAAATATGATACGGGATCCGGAGGAGGAGGA
GCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCACAACAATGGCCAAAGAAATCAAC
40 GGTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGAT
GTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAACAA
CAAACGTCGATGCCAAAGTAAAGCTGCAGAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCC
GCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCCTGAATAAATTGGGAGAAAAATATAACGACATTT
GCTGAAGAGACTAAGACAAATATCGTAAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCC
45 GAAGCATCTAACGATATCGCGGATTTCATTGGATGAACCAACACTAAGGCAGACGAAGCCGTCAAACCGCCCAATGAA
GCCAAACAGCGCGCGAAGAAACCAACGCTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAGCTG
GAAGCTGCCGCTGGCACAGCTAATACTGCAGCGCAGCAAGGCTGCTGCTGCGCAAAAGTTACCGACATCAAAGCT
GATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAACAGTGCCGACGTGTACACCAGGAAGAGTCTGCAGC
AAATTTGTGAGAATTGATGCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCCATT
50 GCCGATCACGATACTCGCTGAACGGTTTGGATAAAACAGTGTACAGCCTGCGCAAAGAAACCGCCAAGGCCTTGCA
GAACAAGCCGCGCTCTCCGGTCTGTTCACCTTACAACGTGGGTCTCGAGCACCACCACCACCACCTGA

1 MSDLANDSFI RQVLDRQHFE PDGKYHLFGS RGLAERSGH IGLGKIQSHQ
51 LGNLMIQQAA IKGNIGYIVR FSDHGHEVHS PFDNHASHSD SDEAGSPVDG
55 FSLYRIHWDG YEHPADGYD GPQGGYPAP KGARDIYSYD IKGVAQNIRL
151 NLTDNRSTGQ RLADRFHNAG SMLTQGVGDG FKRATRYSP E LDRSGNAAEA
201 FNGTADIVKN IIGAAGEIVG AGDAVQGIS E GSNIAVMHGL GLLSTENKMA
251 RINDLADMAQ LKDYAAAAIR DWAVQNPNA QGIEAVSNIF MAAIPIKGIG
301 AVRKYGLGG ITAHPIKRSQ MGAIALPKGK SAVSDNFADA AYAKYPSPYH
10 SRNIRSNLEQ RYKKNITSS TVPPSNGKNV KLADQRHPKT GVPFDGKGF
401 NFEKHVKYDT GSGGGGATND DDVKAATVA IAAAYNNGQE INGFKAGETI
451 YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTV NENKQNVDAK
501 VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKL E ENITTFAEET
551 KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNTKAD EAVKTANEAK
60 QTAETKQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA VAAKVTDIKA
65 DIATNKDNIA KKANSADVYT REESDSKFVR IDGLNATTEK LDTRLASAEK
701 SIADHDTRLN GLDKTVSDLR KETRQGLAEQ AALSGLFPQY NVGLEHHHHH
751 H*

961-ORF46.1

5 ATGGCCACAAACGACGACGATGTTAAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAC
 AAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTCTGAAATAGAAAAGTTAAACAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTTGGGAGAAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
 10 GCCGAAGCATTCAACGATATCGCCGATTCAATTGGATGAAACCAACACTAAGGCAGACGAAGCCCGTCAAAACCGCCAAT
 GAAGCCAAACAGACGGCCGAAGAAACCAACAAACCGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAA
 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAAA
 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGAC
 AGCAAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
 15 ATTGCCGATCAGGATACTCGCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAGAAACCCGCCAAGGCCCTT
 GCAGAACAAGCCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTCTGGTTCAATGTAACGGCTGCAGTCGGCGGC
 TACAAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACTTTGCCGCCAAAGCAGGCGTGGCA
 GTCGGCACTTCGTCGGTCTTCCGCGAGCTTACCATGTCCGGCTCAATTACGAGTGGGGATCCGGAGGAGGAGGATCA
 GATTTGGCAACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTCGAACCCGACGGGAAATACCACTATTC
 20 GGCAGCAGGGGGAACTTGGCAGCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTG
 ATGATTCAACAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCACGGGCACGAAGTCCATTCC
 CCCCTTCGACAACCATGCCTCACATTCCGATCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCCTTTACCGCATC
 CATTGGGACGGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGCGGTATCCCGCTCCCAAAGGC
 GCGAGGGATATATACAGCTACGACATAAAAGGCGTTGCCCAAAATATCCGCTCAACCTGACCGACAACCGCAGCACC
 25 GGACAACGGCTTGGCGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGGACCGGATTCAAACGCGCC
 ACCCGATACAGCCCGGAGCTGGACAGATCGGGCAATGCCGCCGAAGCCTTCAACGGCACTGCAGATATCGTTAAAAAC
 ATCATCGGCGCGGAGGAGAAATTTGTCGGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATTGCTGTC
 ATGCACGGCTTGGGTCTGCTTTCCACCGAAAACAAGATGGCGCGCATCAACGATTGGCAGATATGGCGCAACTCAAA
 GACTATGCCCGCAGCAGCCATCCCGGATTGGGCAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTGAGCAAT
 30 ATCTTTATGGCAGCCATCCCCATCAAAGGATTGGAGCTGTTCCGGGAAAAATACGGCTTGGGCGGCATCAGGCGACAT
 CCTATCAAGCGGTGCGAGATGGGCGCGATCGCATTGCCGAAAGGGAAATCCGCGCTGACGACACAATTTTGCCGATGCG
 GCATACGCCAAATACCCGTCCCTTACCATTCCCGAAATATCCGTTCAAACCTTGGAGCAGCGTTACGGCAAGAAAAAC
 ATCACCTCCTCAACCGTGCCGCGCTCAAACGGCAAAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTA
 35 CCGTTTGACGGTAAAGGGTTTCCGAATTTTGAGAAGCAGCTGAAATATGATACGCTCGAGCACCACCACCACCACCAC
 TGA

1 MATNDDVVK AATVAIAAAY NNGQEINGFK AGETIYDIDE DGTITKKDAT
 51 AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDAKVKAAE SEIEKLTTKL
 101 ADTDAALADT DAALDATTNA LNKLGENTIT FAEETKTNIV KIDEKLEAVA
 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAKVK
 201 AAETAAGKAE AAAGTANTAA DKAEEVAKV TDIKADIATN KDNIAKKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
 301 VSDLRKETRQ GLAEQAALSG LFQPYNVGRF NVTAAVGGYK SESAVAIGTG
 351 FRFTENFAAK AGVAVGTSSG SSAAYHVGVN YEWGSGGGGS DLANDSFIRQ
 401 VLDRQHFEFD GKYHLFSGRG ELAERSGHIG LGKIQSHQLG NLMIQQAIAK
 451 GNIGYIVRFS DHGHEVHSPF DNHASHSDSD EAGSPVDGFS LYRIHWDGYE
 501 HHPADGYDGP QGGGYPAKPG ARDIYSYDIK GVAQNIRLNL TDNRSTGQRL
 551 ADRFHNAGSM LTQVGVDGFK RATRYSPELD RSGNAAEAFN GTADIVKNII
 601 GAAGEIVGAG DAVQGISEGS NIAVMHGLGL LSTENKMARI NDLADMAQLK
 50 651 DYAAAIRDW AVQNPNAOQ IEAVSNIFMA AIPKIGIGAV RGKYGLGGIT
 701 AHPIKRSQMG AIALPKGKSA VSDNFADAAY AKYPSPYHSR NIRSLEQRY
 751 GKENITSSTV PPSNGKNVKL ADQRHPKTGV PFDGKGFPNF EKHVKYDTLE
 801 HHHHHH*

55 961-741

ATGGCCACAAACGACGACGATGTTAAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAC
 60 AAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTCTGAAATAGAAAAGTTAAACAACCAAGTTAGCAGACACTGAT
 TCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCAACCGCTTGAATAAATTTGGGAGAAAAATATAACGACA
 GCTGCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
 GCCGAAGCATTCAACGATATCGCCGATTCAATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCAAT
 GAAGCCAAACAGACGGCCGAAGAAACCAACAAACCGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAA
 65 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAA
 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTCCCGACGTGTACACCAGAGAAGAGTCTGAC
 AGCAAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
 ATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAGAAACCCGCCAAGGCCCTT

GCAGAACAAAGCCGCGCTCTCCGGTCTGTTCACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCGGCGGC
 TACAAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACTTTGCCGCCAAAGCAGGCGTGGCA
 5 GTCCGCACTTCGTCCGGTCTTCCGCAGCCTACCATGTCCGGCGTCAATTACGAGTGGGGATCCGGAGGGGGTGGTGTG
 GCCGCCGACATCGGTGCGGGGCTTGCCGATGCACTAACCGCACCGCTCGACCATAAAGACAAAGGTTTGAGTCTTTG
 ACGCTGGATCAGTCCGTACGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAACTTATGGAACCGGT
 GACAGCCTCAATACGGGCAAATTGAAGAACGACAAGGTGAGCGGTTTCGACTTTATCCGCCAAATCGAAGTGGACGGG
 CAGCTCATTACCTTGAGAGTGGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTCAGACCGAG
 CAAATACAAGATTCCGAGCATTCGGGGAAGATGGTTGCGAAACGCCAGTTTCAAGTTCGGCGACATAGCGGGCGAACAT
 10 ACATCTTTTGACAAGCTTCCCGAAGGCGGCGAGGCGACATATCGCGGACGCGCTTCGGTTTCAGACGATGCCGGCGGA
 AACTGACCTACACCATAGATTTCGCCGCCAAGCAGGGAACGGCAAAATCGAACATTGAAATCGCCAGAACTCAAT
 GTCGACCTGGCCGCCGATATCAAGCCGATGGAACCGCATGCCGTATCAGCGGTTCCGCTCTTTACAACCAA
 GCCGAGAAAGGCGATTACTCCCTCGGTATCTTTGGCGGAAAGCCAGGAAGTTGCGCGCAGCGGAAGTGAAGAAC
 GTAAACGGCATACGCCATATCGGCCTTGCCGCCAAGCAACTCGAGCACCACCACCACCACCTGA

15 1 MATNDDVVK AATVAIAAAY NNGQINGFK AGETIYDIDE DGTITKDAT
 51 AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDKVKAAE SEIEKLTKL
 101 AATDAALADT DAALDATTNA LNKLGENTT FAEETKTIV KIDEKLEAVA
 151 DTVDKHABAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAVK
 201 AAETAAGKAE AAAGTANTAA DKAEVAKV TDIKADIATN KDNIAKKANS
 20 ADVYTRESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
 301 VSDLRKETRQ GLAEQAALSG LFQPYNVGRF NVTAAVGGYK SESAVAIGTG
 351 FRFTENFAAK AGVAVGTSSG SSAAYHVGUN YEWGSGGGV AADIGAGLAD
 401 ALTAPLDHKD KGLQSLTDQ SVRKNEKLK AAQGAERTYG NGDSLNTGKL
 451 KNDKVSFRDF IRQIEVDGQL ITLESGEFQV YKQSHSALTA FQTEQIQDSE
 25 501 HSGKMAVRQ FRIGDIAGEH TSFDKLPPEG RATYRGTAFG SDDAGGKLT
 551 TIDFAAQGN GKIEHLKSP LNVDLAAADI KPDGKRHAVI SGSVLYNQAE
 601 KGSYSLGIFG GKAEVAGSA EVKTVNGIRH IGLAAKQLEH HHHHH*

30 **961-983**
 ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAAC
 35 AAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCAACCGCCTGAATAAATTGGGAGAAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
 GCCGAAGCATTCACGATATCGCCGATTCTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCCAAT
 GAAGCCAAACAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAAA
 40 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAA
 CTTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAACAGTGCCGACGTGTACACGAGAGAAGAGTCTGAC
 AGCAAAATTTGTCAGAAATTGATGGTCTGAACCGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
 ATTGCCGATCACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAGAAACCCGCCAAGGCCCTT
 GCAGAACAAAGCCGCTCTCCGGTCTGTTCACCTTACAACGTGGGTGCGTTCAATGTAACGGCTGCAGTCGGCGGC
 45 TACAAATCCGAATCGGCAGTCGCCATCGGTACCGGCTTCCGCTTTACCGAAAACTTTGCCGCCAAAGCAGGCGTGGCA
 GTCGGCACTTCGTCCGGTCTTCCGCAGCCTACCATGTCCGGCGTCAATTACGAGTGGGGATCCGGCGGAGGCGGCACT
 TCTGCGCCGCACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGGAAATCAGCAGCAGTA
 TCTTACCGCGGTATCAAGAAGAAATGTGCAAGACAGCAAGCATGCTCTGTGCGCGGTGCGGATCGGCTTACGCTTACA
 GACAGGGATGCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTCCAAACCAATGACGCATACAAG
 50 AATTGATCAACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACGCGGGTAGAGGTAGGTATCGTCGACACAGGC
 GAATCCGTCGGCAGCATATCCTTTCCCGAATGTATGGCAGAAAAGAACCGGTATAACGAAAATTACAAAACATAT
 ACGGCGTATATGCGGAAGGAAGCGCTGAAGACGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAGGCCGTT
 ATAGAGACTGAAGCAAAGCCGACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTGGTCTCCCATATTATT
 GCGGGCGTTCCGTGGACGGCAGACCTGCAGGCGGTATTGCGCCGATGCGACGCTACACATAATGAATACGAATGAT
 55 GAAACCAAGAAGCAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAAGCTGGCGTGGCATCGTC
 AATAACAGTTTTGGAACAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTTCGGAGGAGCAGTACCGC
 CAAGCGTTGCTCGACTATTCCGCGCGGTGATAAACAGACGAGGATCCGCTGATGCAACAGAGCGATTACGCAAC
 CTGTCTTACCACATCCGTAATAAAAAATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACA
 TATGCCCTATTGCCATTTTATGAAAAAGACGCTCAAAAGGCATTATCACAGTCGAGGCGTAGACCGCAGTGGAGAA
 60 AAGTTCAAACGGGAAATGTATGGAAACCGGTACAGAACCGCTTGAGTATGGCTCAACCAATTCGGAATTACGCTCC
 ATGTGGTGCTGTCCGCACCCATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTGCCGGAACATCC
 TTTTCCGCACCCATCGTAACCGGCACGGCGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGT
 ACCACGTTGCTGACGACGGCTCAGGACATCGGTGCAGTCGGCGTGGACAGCAAGTTCCGCTGGGGACTGCTGGATGCG
 GGTAAAGCCATGAACGACCGCGTCTTTCCGTTCCGGCGACTTTACCGCGGATACGAAAGGTACATCCGATATTGCC
 65 TACTCTTCCGTAACGACATTTTCAGGACCGGCGGCTGATCAAAAAAGGCGGCGAGCAACTGCAACTGCACGGCAAC
 AACACCTATACGGGCAAAACCATATCGAAGCGGCTGCTGTTGTACGGCAACAAACAAATTCGATTCGCGCTC
 GAAACCAAAGGTGCGCTGATTATAACGGGCGGCATCCGGCGGAGCCTGAACAGCGACGGCATTTGCTATCTGGCA
 GATACCGACCAATCCGGCGCAACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAGGTACGCTGTAC

ACACGTTTGGGCAAACTGCTGAAAGTGGACGGTACGGCGATTATCGGCGGCAAGCTGTACATGTCGGCACGCGGCAAG
 GGGGACGGCTATCTCAACAGTACCGGACGACGTGTTCCCTTCCTGAGTGCCGCCAAAATCGGGCAGGATTATTCTTTC
 TTCACAAACATCGAAACCGACGGCGGCTGCTGGCTTCCTTCGACAGCGTCGAAAAACAGCGGGCAGTGAAGGCGAC
 5 ACGTGTCTTATTATGTCCGTGCGGGCAATGCGGCACGGACTGCTTCGGCAGCGGCACATTCGCGCCCGCCGGTCTG
 AAACACGCCGTAGAACAGGGCGGCAGCAATCTGGAAACCTGATGGTTCGAACCTGGATGCCTCCGAATCATCCGCAACA
 CCCGAGACGGTTGAAACTGCGGCAGCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTCCGCGCA
 GCGGCAGCCGTACAGCATGCGAATGCCGCCGACGGTGTACGCATCTTCAACAGTCTCGCCGTACCGTCTATGCCGAC
 AGTACCGCCGCCCATGCCGATATGCAGGGACGCCGCTGAAAGCCGTATCGGACGGGTGAGCCACAACGGCACGGGT
 10 CTGCGCGTCATCGCGCAACCCAAACAGGACGGTGGAACTGGGAACAGGGCGGTGTGAAGGCAAAATGCGCGGCAGT
 ACCCAAACCGTCCGCATTGCCCGGAAAACCGGCGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGCGACACA
 TGGAGCGAAAACAGTGCAAATGCAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCAGCATGCGGGCGATATC
 GGCATATCTCAAAGCCCTGTTCTCCTACGGACGCTACAAAAACAGCATACGCCCGCAGCACCCGTCGGGACGAACATGCG
 GAAGGCAGCGTCAACGGCACGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGGCGCAACGGGAGAT
 15 TTGACGGTGAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCCGCCGAAAAGGCAGTGCTTTGGGCTGG
 AGCGGCAACAGCCTCACTGAAGGCACGCTGGTGGACTCGCGGTCTGAAGCTGTCGCAACCCCTTGAGCGATAAAGCC
 GTCCGTTTGAACGGCGGGCGTGGAAACGCGACCTGAACGGACGCGACTACACGGTAACGGGCGGCTTTACCGGCGCG
 ACTGCAGCAACCGGCAAGACGGGGGCACGCAATATGCCGCACACCCGCTCTGTTGCGCGCTGGGCGCGGATGTCGAA
 TTCGGCAACGGCTGGAACGGCTTGGCAGTTACAGTACGCTACGCGGTTCCAAACAGTACGGCAACCACAGCGGACGAGTC
 GCGTAGGCTACCGGTTCTCGAGCACCACCACCACCACCTGA

20
 1 MATNDDDVKK AATVAIAAAY NNGQEINGFK AGETIYDIDE DGTITKRDAT
 51 AADVEADDFK GLGLKKVVTN LTKTVNENKQ NVDAKVKAAE SEIEKLTTKL
 101 ADTDAALADT DAALDATTNA LNKLGENTIT FAETRTNIV KIDEKLEAVA
 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAEB TKQNVDAKVK
 25 201 AAETAAGKAE AAAGTANTAA DKAEAVAAKV TDIKADIATN KDNIKKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
 301 VSDLRKETRQ GLAEQAALSG LFQPYNVGRF NVTAAVGGYK SESAVAIGTG
 351 FRFTENFAAK AGVAVGTSSG SSAAYHVGUN YEWGSGGGGT SAPDFNAGGT
 401 GIGSNSRATT AKSAAVSYAG IKNEMCKDRS MLCAGRDDVA VTDRDAKINA
 30 451 PPPNLHTGDF PNPNDAYKNL INLKPAIEAG YTGRGVEVGI VDTGESVSGSI
 501 SFPELYGRKE HGVNENYKNY TAYMRKEAPE DGGGKDIEAS FDDEAVIETE
 551 AKPTDIRHVK EIGHIDLVS H IIGGRSVDGR PAGGIAPDAT LHIMNTNDET
 601 KNEMMVAAIR NAWVKLGERG VRIVNNSFGT TSRAGTADLF QIANSEEQYR
 651 QALLDYSGGD KTDEGIRLMQ QSDYGNLSYH IRNKNMLFIF STGNDAQAQP
 35 701 NITYALLPFYE KDAQKGITV AGVDRSGEKF KREMYGEPGT EPLEYGSNHC
 751 GITAMWCLSA PYEASVRFTR TNPIQIAGTS FSAPIVTGTA ALLLQKYPWM
 801 SNDNLRITLL TTAQDIGAVG VDSKFGWLL DAGKAMNGPA SFPFGDTAD
 851 TKGTSDIAYS FRNDISGTGG LIKKGGSQQLQ LHGNNTYTGK TIEGGSVLV
 901 YGNNSKDMRV ETKGALITNG AASGGSLSND GIVYLADTDQ SGANETVHIK
 40 951 GSLQLDGKGT LYTRLGKLLK VDGTAIIGK LYMSARGKGA GYLNSTGRRV
 1001 PFLSAAKIGQ DYSFTNIET DGLLASLDS VEKTAGSEGD TLSYVYVRGN
 1051 AARTASAAAH SAPAGLKHAV EQGGSNLENL MVELDASESS ATPETVETAA
 1101 ADRTDMPGIR PYGATFRAAA AVQHANAADG VRIFNSLAAT VYADSTAHA
 1151 DMQGRRLKAV SDGLDHNGTG LRVIAQTQOD GGTWEQGGVE GKMRGSTQTV
 45 1201 GIAAKTGENT TAAATLGMGR STWSSENSANA KTDSSISLFA IRHDAGDIGY
 1251 LKGLFSYGRY KNSISRSTGA DEHAEGSVNG TLMQLGALGG VNPVFAATGD
 1301 LTVEGGRLYD LLKQDAFAEK GSALGWSGNS LTEGTLVGLA GLKLSQPLSD
 1351 KAVLFATAGV ERDLNGRDYT VTGGFTGATA ATGKTGARNM PHTRLVAGLG
 1401 ADVEFGNGWN GLARYSYAGS KQYGNHSGRV GVGYRFLEHH HHHH*

50

961c-ORF46.1

ATGGCCACAAACGACGACGATGTTAAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCC
 55 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAC
 AAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAATCTGAAATAGAAAAGTTAAACAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTTGGGAGAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTTCGACAAGCAT
 CCCGAGCATTTCAACGATATCGCCGATTCTTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCAAT
 60 GAAGCCAAACAGACGGCCGAAGAAACCAAAACAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAA
 GCCGAAGCTGCCGTGGCAGCTAATACTGCAGCCGACAAGCCGAAGCTGTCGCTGCAAAAGTTACCGACATCAAA
 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAAGCAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGAC
 AGCAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTGCTTCTGCTGAAAAATCC
 65 ATTGCCGATCAGGATACGCTGAAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAGAAACCCGCAAGGCCTT
 GCAAGCAAGCCGCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGGATCCGGAGGAGGAGGATCAGATTTGGCA
 AACGATTCTTTTATCCGGCAGGTTCTCGACCGTCAGCATTTTCGAACCCGACGGGAAATACCACCTATTCGGCAGCAGG
 GGGGAACCTGCCGAGCGCAGCGGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTGATGATTCAA

5 CAGGCGGCCATTAAAGGAAATATCGGCTACATTGTCCGCTTTTCCGATCACGGGCACGAAGTCCATTCCCCCTTCGAC
 AACCATGCGCTCACATTCCGATTCTGATGAAGCCGGTAGTCCCGTTGACGGATTAGCCTTTACCGCATCCATTGGGAC
 GGATACGAACACCATCCCGCCGACGGCTATGACGGGCCACAGGGCGGGCTATCCCGCTCCCAAAGGCGCGAGGGAT
 10 ATATACGACTACGACATAAAAGGCGTTGCCAAAATATCCGCTCAACCTGACCGACAACCCGAGCACCAGACAACGG
 CTGCGGACCGTTTCCACAATGCCGGTAGTATGCTGACGCAAGGAGTAGGCGACGGATTCAAACGCGCCACCCGATAC
 AGCCCCGAGCTGGACAGATCGGGCAATGCCGCCGAGCCTTCAACGGCACTGCAGATATCGTTAAAAACATCATCGGC
 GCGGCAGGAGAAATTGTCGGCGCAGGCGATGCCGTGACGGGCATAAGCGAAGGCTCAAACATTGCTGTATGCACGGC
 TTGGGTCTGCTTTCCACCAGAAAACAAGATGGCGCGCATCAACGATTGTCAGATATGGCGCAACTCAAAGACTATGCC
 15 GCAGCAGCCATCCGCGATTGGGCAGTCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTCAGCAATATCTTTATG
 GCAGCCATCCCCATCAAAGGATTGGAGCTGTTCCGGGAAAATACGGCTTGGGCGGCATCACGGCACATCCTATCAAG
 CGGTCGCAGATGGGCGCATCGCATTGCCGAAAGGAAATCCGCGTCAGCGACAATTTTGGCGATGCGGCATACGCC
 AAATACCCGTCCTTTTACCATTCCCGAAATATCCGTTCAAACTTGGAGCAGCGTTACGGCAAGAAAACATCACCTCC
 TCAACCGTGCCGCGCTCAAACGGCAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTACCGTTTGAC
 GGTAAAGGTTTCCGAATTTTGAAGACAGTGAAATATGATACGCTCGAGCACCACCACCACCACCTGA

1 MATNDDVKK AATVAIAAAY NNGQEINGFK AGETIYDIDE DGTITKKDAT
 51 AADVEADDFK GLGLKKVVTN LTKTVNENKQ NVDKVKAAE SEIEKLTTKL
 101 ADTDAALADT DAALDATNA LNKLGENTT FAEETKTIV KIDEKLEAVA
 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAKVK
 201 AAETAAGKAE AAAGTANTAA DKAEVAKV TDIKADIATN KDNIAKKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
 301 VSDLRKETRO GLAEQAALSG LFQPYNVGGS GGGGSDLAND SFIRQVLDRO
 351 HFEPDGKYLH FGSRGELAER SGHIGLGIQ SHQLGNLMIQ QAAIKGNIGY
 401 IVRFSDHGHE VHSPFDNHAS HSDEAGSP VDGFSLYRIH WDGYEHHPAD
 251 451 GYDGPQGGY PAPKGARDY SYDIKVAQN IRLNLDNRS TQRLADRFH
 501 NAGSMLTQGV GDGFKRATRY SPELDRSNA AEAFTGTADI VKNIIAAGE
 551 IVGAGDAVQG ISEGSNIAMV HGLGLLSTEN KMARINDLAD MAQLKDYAAA
 601 AIRDWAVQNP NAAQIEAVS NIFMAAPIK GIGAVRGKYG LGGITAHPIK
 651 RSQMGALALP KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI
 301 701 TSSTVPPSNG KNVKLADQRH PKTGVPFDGK GPFNFKEHVK YDTLEHHHHH
 751 H*

35 **961c-741**
 ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGTCTGGGTCTGAAAAAGTCTGTGACTAACCTGACCAAAACCGTCAATGAAAAC
 40 AAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGTAAGTAGAAAAGTTAAACAACCAAGTTAGCAGACACTGAT
 CCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
 GCCGAAGCATTCAACGATATCGCCGATTCTTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAACCCGCCAAT
 GAAGCCAAACAGACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAA
 GCCGAAGCTGCCGCTGGCACAGCTAATACTGCAGCCGACAAGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAA
 45 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAACAGTGCCGACGTTGTACACCAGAGAAGAGTCTGAC
 AGCAAAATTTGTCAGAAATGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCC
 ATTGCCGATACGATACTCGCCTGAACGTTTGGATAAAAACAGCTGTACAGCTGCGCAAGAAACCCGCCAAGGCCTT
 GCAGAACAAAGCCGCTCTCGGCTGTTTCAACCTTACAACGTTGGGTGGATCCGGAGGGGGTGGTGTCCGCCCGGAC
 ATCGGTGCGGGGCTTCCGATGCACTAACCGCACCGCTCGACCATAAAGACAAAGGTTTGCAGTCTTTGACGCTGGAT
 50 CAGTCCGTCAGGAAAAACGAGAACTGAAGCTGGCGGCACAAGGTGCGGAAAAAATTATGGAACGGTGACAGCCTC
 AATACGGGCAATTTGAAGAACGACAAGGTGAGCCGTTTTCGACTTTATCCGCCAAATCGAAGTGGACGGGCAGCTCATT
 ACCTTGGAGAGTGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCTTAAACCGCTTTTCAGACCGAGCAATACAA
 GATTCGGAGCATTCCGGGAAGATGGTTGCGAAACGCCAGTTTCAAGATCGGCGACATAGCGGGCGAACATACATCTTTT
 GACAAGCTTCCGAAGGCGGCAGGGCGACATATCGCGGGACGCGTTCCGGTTCAGACGATGCCGGCGGAAAACTGACC
 55 TACACCATAGATTTCCCGCCAAAGCAGGGAACGGCAAAATCGAACATTGAAATCGCCAGAACTCAATGTCGACCTG
 GCCGCCCGGATATCAAGCCGGATGGAACCGCATGCCGTATCAGCGGTTCCGTCTTTACAACCAAGCCGAGAAA
 GGCAGTTACTCCCTCGGTATCTTTGGCGGAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTGAACCCGTAAACGGC
 ATACGCCATATCGGCCTTGCCGCCAAGCAACTCGAGCACCACCACCACCACCTGA

60 1 MATNDDVKK AATVAIAAAY NNGQEINGFK AGETIYDIDE DGTITKKDAT
 51 AADVEADDFK GLGLKKVVTN LTKTVNENKQ NVDKVKAAE SEIEKLTTKL
 101 ADTDAALADT DAALDATNA LNKLGENTT FAEETKTIV KIDEKLEAVA
 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE TKQNVDAKVK
 201 AAETAAGKAE AAAGTANTAA DKAEVAKV TDIKADIATN KDNIAKKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT
 65 301 VSDLRKETRO GLAEQAALSG LFQPYNVGGS GGGVADIG AGLADALTAP
 351 LDHKDKGLQS LTLDQSVRKN EKLKLAQGA EKYNGNDSL NTGKLKNDKV
 401 SRFDFIRQIE VDGQLITLES GEFQVYKQSH SALTAFTQEQ IQDSEHSGKM

-31-

451 VAKRQFRIGD IAGEHTSFDK LPEGGRATYR GTAFGSDDAG GKLTYTIDFA
 501 AKQGNKIEH LKSPELNVDL AAADIKPDGK RHAVISGSVL YNQAEGKSYS
 551 LGIFGGKAQE VAGSAEVKTV NGIRHIGLAA KQLEHHHHHH *

5

961c-983

ATGGCCACAAACGACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATC
 AACGGTTTCAAAGCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATPACCAAAAAAGACGCAACTGCAGCC
 GATGTTGAAGCCGACGACTTTAAAGGTCTGGGTCTGAAAAAGTCTGACTAACCTGACCAAAACCGTCAATGAAAC
 AAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAAATCTGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGAT
 GCCGCTTTAGCAGATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTTGGGAGAAAATATAACGACA
 TTTGCTGAAGAGACTAAGACAAATATCGTAAAAATTTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCAT
 GCCGAAGCATTTCAACGATATCGCCGATTCTTGGATGAAACCAACACTAAGGCAGACGAAAGCCGTCAAAACCGCCAAT
 GAAGCCAAACAGACGGCCGAAGAAACCAACAAACGTCGATGCCAAAGTAAAGCTGCAGAAACTGCAGCAGGCAAA
 GCCGAAGCTGCCGCTGGCAGACTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAA
 GCTGATATCGCTACGAACAAAGATAATATTGCTAAAAAGCAACAGTGCCGACGTGTACACCAGAGAAGAGTCTGAC
 AGCAAAATTTGTCAGAATTGATGGTCTGAACGCTACTACCGAAAAATTTGGACACACGCTTGGCTTCTGCTGAAAAATCC
 ATTGCCGATACGATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAGAAACCCGCCAAGGCCTT
 GCAGAACAAGCCGCGCTCTCCGGTCTGTTCACCTTTACAACGTGGGTGGATCCGGCGAGGCGGCACCTTCTCGCCCC
 GACTTCAATGCAGGCGGTACCGGTATCGGCAGCAACAGCAGAGCAACAACAGCGAAATCAGCAGCAGTATCTTACGCC
 GGTATCAAGAACGAAATGTCAAAGACAGAAGCATGCTCTGTGCCGGTCCGGATGACGTTTGGGTTACAGACAGGGAT
 GCCAAAATCAATGCCCCCCCCCGAATCTGCATACCGGAGACTTTTCCAAACCCAAATGACGCATACAAGAAATTTGATC
 AACCTCAAACCTGCAATTGAAGCAGGCTATACAGGACCGCGGGTAGAGGTAGGTATCGTCGACACAGGCGAATCCGTC
 AGCAGCATATCTTTCCGAAGCTGATGCGAGAAAAGAACACGGCTATAACGAAATTTACAAAACTATACGGCGTAT
 ATGCGGAAGGAAGCGCCTGAAGACGGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAGGCCGTTTATGAGACT
 GAAGCAAAGCCGACGGATATCCGCCACGTAAAGAAATCGGACACATCGATTGGTCTCCCATATTATTGGCGGGCGT
 TCCGTGGACGCGCAGACCTGCAGGCGGTATTGCGCCGATGCGACGCTACACATAATGAATACGAATGATGAAACCAAG
 AACGAAATGATGGTTGCAGCCATCCGCAATGCATGGGTCAAGCTGGGCGAACGTGGCGTGGCGCATCGTCAATAACAGT
 TTTGGAACAACATCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTCGGAGGAGCAGTACCGCCAAGCGTTG
 CACATCCGTAATAAAAAACATGCTTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACATATGCCCTA
 TTGCCATTTTATGAAAAAGACGCTCAAAAAGGCATTATCACAGTCGAGGCGTAGACCGCAGTGGAGAAAAGTTCAA
 CGGAAATGTATGGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTTGCGGAATTACTGCCATGTGGTGC
 CTGTGCGCACCTTATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTTGCCGAACATCTTTTCCGCA
 CCCATCGTAACCGGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGTACCACGTTG
 CTGACGACGGCTCAGGACATCGGTGCGAGTCCGGCTGAGACAGCAAGTTCCGGCTGGGGACTGCTGGATCGGGTAAGGCC
 ATGAACGGACCCGCGTCTTTCCGTTCCGGCGACTTTACCGCCGATACGAAAGGTACATCCGATATTGCTACTCCTTC
 CGTAACGACATTTTACGGCACGGCGGCTGATCAAAAAGGCGGCAGCCAACCTGCAACTGCACGGCAACAACACCTAT
 ACGGGCAAAACCATTTATCGAAGGCGGTTGCTGGTGTGTGACGGCAACAACAATCGGATATGCGCGTGGAAACCAAA
 GGTGCGCTGATTTATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCGACGGCATTTGCTATCTGGCAGATACCGAC
 CAATCCGGCGCAACGAAACCGTACACATCAAAGGCAGTCTGACGCTGGACGGCAAAGGTACGCTGTACACAGCTTTG
 GGCAAACTGCTGAAAGTGGACGGTACGGCGATTATCGGCGGCAAGCTGTACATGTCGGCGACGGCAAGGGGGCAGGC
 TATCTCAACAGTACCGGACGACGTGTTCCTTCTGAGTGCCGCCAAAATCGGGCAGGATTATTCTTTCTTCAAAAC
 ATCGAAACCGACGGCGGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAAGGGACACGCTGTCC
 TATTATGTCCGTGCGCGCAATGCGGCACGGAATGCTTCCGGCAGCGGCACATTCGCGCGCCCGCGGTGTGAAACACGCC
 GTAGAACAGGGCGGCAGCAATCTGGAACCTGATGGTGAACCTGGATGCCATCCGAATCATCCGCAACACCCGAGACG
 GTTGAACCTGCGGCAGCGACCGACAGATATCCGGGCTACCGCCCTACGGCGCAACTTTCCGCGCAGCGGCAGCC
 GTACAGCATGCGAATGCCGCCGACGGTGTACGCACTTCAACAGTCTCGCCGCTACCGCTATGTCGCGACAGTACCGCC
 GCCCATGCGGATATGCAGGACGCGCCCTGAAAGCCGTATCGGACGGGTGGACCACAACGGCAGCGGTCTGCGCGTC
 ATCGCGCAAAACCAACAGGACGGTGAACGTGGGAACAGGGCGGTGTTGAAGGCAAAATGCGCGGCAGTACCCAAACC
 GTCGGCATTTCCGCGAAAACCGGCGAAAATACGACAGCAGCCGCCACACTGGGCATGGGACGACGACATGGAGCGAA
 AACAGTGAATGCAAAAACCGACAGCATTAGTCTGTTTGCAGGCATACGGCAGATGCGGGCGATATCGGCTATCTC
 AAAGGCCGTGTTCTCTACGGACGCTACAAAAACAGCATCAGCCGACGACCGGTGCGGACGAACATGCGGAAGGCAGC
 GTCAACGGCAGCTGATGCAGCTGGGCGCACTGGGCGGTGTCAACGTTCCGTTTGGCGCAACGGGAGATTTGACGGTC
 GAAGGCGGTCTGCGCTACGACCTGCTCAAACAGGATGCATTCGCCGAAAAAGGCAGTGTCTTGGGCTGGAGCGGCAAC
 AGCCTCACTGAAGGCACGCTGGTTCGACTCGCGGTCTGAAGCTGTGCAACCCCTTACGCGATAAAGCCGTCTGTTT
 GCAACGGCGGGCGTGAACGCGACCTGAACGGACGCGACTACACGGTAACGGCGGCTTTACCGGCGGACTGCAGCA
 ACCGGCAAGACGGGGCACGCAATATGCCGCACACCCGCTGGTTGCGGCGCTGGGCGCGGATGTGCAATTCGGCAAC
 GGCTGGAACGGCTTGGCACGTTACGCGTACGCCGTTTCCAACAGTACGGCAACCACAGCGGACGAGTGGCGTAGGC
 TACCGGTTCTCGAGCACCACCACCACCACCTGA

1 MATNDDVVK AATVAIAAY NNGQEINGFK AGETIYDIDE DGTITKDAT
 51 AADVEADDFK GLGLKKVVTN LTKTVNENKQ NVDKVKAAE SEIEKLPTKL
 101 ADTDAALADT DAALDATNA LNKLGENTIT FAETKTIV KIDEKLEAVA
 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAEB TKQNVDAKVK
 201 AAETAAGKAE AAAGTANTAA DKAEVAARKV TDIKADIATN KDNIKAKANS
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT

65

-32-

301 VSDLRKETRO GLAEQAALSG LFQPYNVGGS GGGGTSAPDF NAGGTGIGSN
 351 SRATTAKSAA VSYAGIKNEM CKDRSMLCAG RDDVAVTDRD AKINAPFPNL
 401 HTGDFPNPND AYKNLINLKP ATEAGYTGRG VEVGIVDTGE SVGSI SFPEL
 5 451 YGRKEHGYNE NYKNYTAYMR KEAPEDGGGK DIEASFDEA VIETEARPTD
 501 IRHVKEIGHI DLVSHIIGGR SVDGRPAGGI APDATLHIMN TNDETKNEMM
 551 VAAIRNAWVK LGERVIRVN NSFGTTSRAG TADLFQIANS EEQYRQALLD
 601 YSGGDKTDEG IRLMQQSDYG NLSYHIRNKN MLFIFSTGND AQAQPNFYAL
 651 LPFYEKDAQK GIITVAGVDR SGEKFKREMY GEPGTEPLEY GSNHCGITAM
 701 WCLSAPYEAS VRFTRTNPIQ IAGTSFSAPI VTGTAALLLQ KYPWMSNDNL
 10 751 RTTLLTTAQD IGAUVGDSKF GWGLLDAGKA MNGPASFPFG DFTADTKGTS
 801 DIAYSFRNDI SGTGGLIKKG GSQQLQHGNV TYTGKTIIEG GSVLYYGNMK
 851 SDMRVETKGA LIYNGAASGG SLNSDGIIVL ADTDQSGANE TVHIKGSLLQ
 901 DGKGTLYTRL GKLLKVDGTA IIGGKLYMSA RKGAGYLSN TGRRVFFLSA
 951 AKIGQDYSFF TNIEDGGLL ASLDSVEKTA GSEGDLSY VRRGNAARTA
 15 1001 SAAAHSAFAG LKHAVERQGS NLENLMVELD ASESSATPET VETAAADRTD
 1051 MPGIRPYGAT FRAAAAVQHA NAADGVRIFN SLAATVYADS TAAHADMOGR
 1101 RLKAVSDGLD HNGTGLRVIA QTQDGGTWE QGGVEGKMRG STQTVGLAAK
 1151 TGENTTAAAT LGMGRSTWSE NSANAKTDSI SLFAGIRHDA GDIGYLGKLF
 1201 SYGRYKNSIS RSTGADEHAE GSVNGTLMQL GALGGVNVFF AATGDLTVEG
 20 1251 GLRYDLKQD AFAEKGSALG WSGNSLTEGT LVGLAGLKL QPLSDKAVLF
 1301 ATAGVERDLN GRDYTVTGGF TGATAATGKT GARNMPHTRL VAGLGADVEF
 1351 GNGWNLARY SYAGSKQYGN HSGRVGVGYR FLEHHHHHH*

25

961cL-ORF46.1

ATGAAACACTTTCCATCCAAAGTACTGACCACAGCCATCCTTGCCACTTCTGTAGCGGCGCACTGGCAGCCACAAAC
 GACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAACAATGGCCAAGAAATCAACGGTTTCAA
 GCTGGAGAGACCATCTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCC
 30 GACGACTTTAAAGGTCTGGGTCTGAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAACAAACAAACGTC
 GATGCCAAAGTAAAGCTGCAGAACTGAAATAGAAAAGTTAAACAACCAAGTTAGCAGACACATGATGCCGCTTTAGCA
 GATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAAATATAACGACATTTGCTGAAGAG
 ACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTC
 AACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGACAGACGAAGCCGTCAAACCGCCAAATGAAGCCAAACAG
 35 ACGGCCGAAGAAACCAACAAACCGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAGCCGAAGCTGCC
 GCTGGCACAGCTAATACTGCAGCCGACAAGCCGAAGCTGCTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCT
 ACGAACAAAGATAATATTGCTAAAAAGCAACAGTGGCGACGTGTACACAGAGAAGAGTCTGACAGCAAATTTGTC
 AGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATCAC
 GATACTCGCCTGAACGGTTTGGATAAAACAGTGTGACACCTGCGCAAAGAAACCCGCCAAGGCCCTTCGAGAACAAGCC
 40 GCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGGATCCGGAGGAGGAGGATCAGATTTGGCAAACGATTCCTTTT
 ATCCGGCAGGTTCTCGACCGTCAGCATTTGCAACCCGACGGGAAATACCACCTATTCGGCAGCAGGGGGAACTTGCC
 GATCGCAGCGCCATATCGGATTGGGAAAAATACAAAGCCATCAGTTGGGCAACCTGATGATTCAACAGGCGGCCATT
 AAAGGAAATATCGGCTACATTGTCGCTTTTCCGATCAGGGGCACGAAGTCCATTCCCCCTTCGACAACCATGCCCTCA
 CATTCGATTTCTGATGAAGCCGCTAGTCCCGTTGACGGATTAGCCTTTACCGCATCCATTGGGACGGATACGAACAC
 45 CATCCGCGGACGGCTATGACGGGCCACAGGGCGGGCGGTATCCCGCTCCCAAAGGCGGAGGGATATATACAGCTAC
 GACATAAAAGCGGTTGCCCAAATATCCGCCTCAACCTGACCGACAACCGCAGCACCAGGACAACGGCTTGCCGACCGT
 TTCCACAATGCCGGTAGTATGCTGACGCAAGGATAGGCGACGGATTCAAACGCGCCACCCGATACAGCCCCGAGCTG
 GACAGATCGGGCAATGCCGCGGAGCCTTCAACGGCAGCTGCAGATATCGTTAAAAACATCATCGGCGCGGAGGAGAA
 ATTGTCGGCGCAGGCGATGCCGTGCAGGGCATAAGCGAAGGCTCAAACATTGCTGTATGCACGGCTTGGGTCTGCTT
 50 TCCACCGAAAACAAGATGGCGCGCATCAACGATTTGGCAGATATGGCGCAACTCAAAGACTATGCCGCGCAGGCCATC
 CGCGATTGGGCGATCCAAAACCCCAATGCCGCACAAGGCATAGAAGCCGTGAGCAATATCTTTATGGCAGCCATCCCC
 ATCAAAGGGATTGGAGCTGTTCCGGGAAAAATACGGCTTGGGCGGCATCAGGCACATCCTATCAAGCGGTGCGAGATG
 GCGCGATCGCATTGCCGAAAGGAAATCCGCCGTGACGACAATTTTGGCGATGCGGCATACGCCAAATACCCGTCC
 CCTTACCATTTCCGAAATATCCGTTCAAACCTGGAGCAGCGTTACGGCAAAGAAAACATCACCTCTCAACCGTGCCG
 55 CCGTCAAACGGCAAAAATGTCAAACCTGGCAGACCAACGCCACCCGAAGACAGGCGTACCGTTTACGGTAAAGGGTTT
 CCGAATTTTGAAGACAGTGAATATGATACGTAACCTCGAG

55

1 MKHFPSKVL TAILATFCSG ALAATNDDDV KKAATVAIAA AYNNGQING
 51 FKAGETIYDI DEDGTITKD ATAADVEADD FKGLGLKKVV TNLTKTVNEN
 101 KQNVDAKVA AESEIEKLTT KLADTDAALA DTDALDATT NALNLGENI
 60 151 TTFABETKTN IVKIDEKLEA VADTVDKHAE AFNDIADSLD ETNTKADEAV
 201 KTANEAKQTA EETKQNVDAK VKAAETAAGK AEAAAGTANT AADKAEAVAA
 251 KVTDIKADIA TNKDNIAKKA NSADVYTREE SDSKFVRIDG LNATTEKLDL
 301 RLASAEKSIA DHDTRLNGLD KTVSDLRKET RQGLAEQAAL SGLFQPYNVG
 351 GSGGGGSDLA NDSFIRQVLD RQHFEPDGKY HLFGRGELA ERSGHIGLGK
 65 401 IQSHQLGNLM IQQAAIKGNI GYIVRFSHDG HEVHSPFDNH ASHSDSDEAG
 451 SPVDGFSLYR IHWDGYEHHP ADGYDGPQGG GYPAPKGARD IYSYDIKQVA
 501 QNIRLNLTDN RSTGQRLADR FHNAGSMLTQ GVGDFGKRAF RYSPELDMSG

551 NAAEAFNGTA DIVKNIIGAA GEIVGAGDAV QGISEGSNIA VMHGLGILLST
 601 ENKMARINDL ADMAQLKDYA AAAIRDWAVQ NPNAAQGIEA VSNIFMAAIP
 651 IKGIGAVRGK YGLGGITAHF IKRSQMGAI A LPKGKSAVSD NFADAAYAKY
 701 PSPYHSRNIR SNLEQRYGKE NITSSTVPPS NGKNVKLADQ RHPKTGVFPD
 751 KGKFPNFEKH VKYDT*

961cL-741

10 ATGAAACACTTTCCATCCAAAGTACTGACCACAGCCATCCTTGCCACTTCTGTAGCGGCGCACTGGCAGCCACAAAC
 GACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAAACATGGCCAAAGAAATCAACGGTTTCAAA
 GCTGGAGAGACCATTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCC
 GACGACTTTAAAGGCTCGGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAACAACAAAACGCTC
 15 GATGCCAAAGTAAAAGCTGCAGAACTCGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCA
 GATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAATATAACGACATTTGCTGAAGAG
 ACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTC
 AACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCAATGAAGCCAAACAG
 ACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAAGCCGAAGCTGCC
 20 CTTGGCAGAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCT
 ACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCAGCAGCTGTACACCAGAGAAGAGTCTGACAGCAAAATTTGTC
 AGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATCAC
 GATACTCCGCTGAACGGTTTGGATAAAACAGTGTGAGACCTGCGCAAGAAACCCGCCAAGGCCCTTCGAGAACAAGCC
 GCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGGATCCGGAGGGGGTGGTGTGCGCCGCGACATCGGTGCCGGG
 25 CTTGCCGATGCACTAACCGCACCGCTCGACCATAAAGACAAGGTTTGCAGTCTTTGACGCTGGATCAGTCCGTCAGG
 AAAAACGAGAACTGAAGCTGGCGGCACAAGGTCGGGAAAAAATTTATGGAACGGTGACAGCCTCAATACGGGCAAA
 TTGAAGAACGACAAGGTCAGCCGTTTCGACTTTATCCGCCAAATCGAAGTGACGGGCAGCTCATTACCTTGGAGAGT
 GGAGAGTTCCAAGTATACAAACAAAGCCATTCCGCCTTAACCGCCTTTCAGACCGAGCAAAATACAAGATTCCGAGCAT
 TCCGGGAAGATGGTTGCGAAACGCCAGTTTCAAGATCGCGCAGATAGCGGGCGAACATACATCTTTTGACAAGCTTCCC
 GAAGGCGGCGAGGGCGACATATCGCGGGACGGGCTTCGGTTTCAAGCATGCCGGCGGAAACTGACCTACACCATAGAT
 30 TTCGCGCCAAGCAGGGAACGGCAAAATCGAACATTTGAAATCGCCAGAACTCAATGTCGACCTGGCCGCGCCGAT
 ATCAAGCCGGATGGAACCGCATGCGGTCTATCAGCGGTTCCGTCCTTTACAACCAAGCCGAGAAAGGCAGTTACTCC
 CTCGGTATCTTTGGCGGAAAAGCCAGGAAGTTGCCGGCAGCGCGGAAGTGAAACCGCTAAACGGCATACGCCCATATC
 GGCCTTGGCGCAAGCAACTCGAGCACCACCACCACCACCTGA

35 1 MKHFPKSVLT TAILATFCSG ALAATNDDDV KKAATVAIAA AYNNGQEING
 51 FKAGETIYDI DEDGTITKRD ATAADVEADD FKGLGLKVV TNLTKTVNE
 101 KQNVDAKVA AESEIEKLTT KLADTDALA DTDALDATT NALNKLGENI
 151 TTFAEETKTN IVKIDEKLEA VADTVDKHAE AFNDIADSLD ETNTKADEAV
 201 KTANEAKQTA BETKQNVDAK VKAAETAAGK AEAAAGTANT AADKAEAVAA
 251 KVTDIKADIA TNKDNIAKKA NSADVYTREE SDSKFVRIDG LNATTEKLD
 40 301 RLSAEKSIA DHDTRLNGLD KTVSDLRKET RQGLAEQAAL SGLFPQYVNG
 351 GSGGGVVAAD IGAGLADALT APLDHKDKGL QSLTLDQSVR KNEKLKLAQ
 401 GAEKTYGNGD SLNTGKLKND KVSFRDFIRQ IEVDGQLITL ESGEFQVYKQ
 451 SHSALTAFQT EQIQDSEHSG KMAKROFRI GDIAGEHTSF DKLPEGGGRAT
 501 YRGTAFGSDD AGGKLYTID FAAKQNGKI EHLKSPBLNV DLAAADIKPD
 45 551 GKRHAIVSGS VLYNQAEKGS YSLGIFGGKA QEVAGSAEVK TVNGIRHIGL
 601 AAKQLEHHHH HH*

961cL-983

50 ATGAAACACTTTCCATCCAAAGTACTGACCACAGCCATCCTTGCCACTTCTGTAGCGGCGCACTGGCAGCCACAAAC
 GACGACGATGTTAAAAAGCTGCCACTGTGGCCATTGCTGCTGCCTACAAACATGGCCAAAGAAATCAACGGTTTCAAA
 GCTGGAGAGACCATTACGACATTGATGAAGACGGCACAATTACCAAAAAAGACGCAACTGCAGCCGATGTTGAAGCC
 GACGACTTTAAAGGCTCGGGTCTGAAAAAAGTCGTGACTAACCTGACCAAAACCGTCAATGAAAAACAACAAAACGCTC
 55 GATGCCAAAGTAAAAGCTGCAGAACTCGAAATAGAAAAGTTAACAACCAAGTTAGCAGACACTGATGCCGCTTTAGCA
 GATACTGATGCCGCTCTGGATGCAACCACCAACGCCTTGAATAAATTGGGAGAAAATATAACGACATTTGCTGAAGAG
 ACTAAGACAAATATCGTAAAAATTGATGAAAAATTAGAAGCCGTGGCTGATACCGTCGACAAGCATGCCGAAGCATTC
 AACGATATCGCCGATTCATTGGATGAAACCAACACTAAGGCAGACGAAGCCGTCAAAACCGCCAATGAAGCCAAACAG
 ACGGCCGAAGAAACCAACAAAACGTCGATGCCAAAGTAAAGCTGCAGAACTGCAGCAGGCAAAGCCGAAGCTGCC
 60 CTTGGCAGAGCTAATACTGCAGCCGACAAGGCCGAAGCTGTGCTGCAAAAGTTACCGACATCAAAGCTGATATCGCT
 ACGAACAAAGATAATATTGCTAAAAAAGCAAACAGTGCAGCAGCTGTACACCAGAGAAGAGTCTGACAGCAAAATTTGTC
 AGAATTGATGGTCTGAACGCTACTACCGAAAAATTGGACACACGCTTGGCTTCTGCTGAAAAATCCATTGCCGATCAC
 GATACTCCGCTGAACGGTTTGGATAAAACAGTGTGAGACCTGCGCAAGAAACCCGCCAAGGCCCTTCGAGAACAAGCC
 GCGCTCTCCGGTCTGTTCCAACCTTACAACGTGGGTGGATCCGGCGGAGGCGGCACCTTCTGCGCCCGACTTCAATGCA
 65 GCGGTACCGGTATCGGCAGCAACAGCAGCAACAACAGCGAAATCAGCAGCAGTATCTTACGCGCGGTATCAAGAAC
 GAAATGTGCGCAAGACAGAAAGCATGCTGTGCGGTGCGGATGACGTTGCGGTTACAGCAGGGATGCCAAATCAAT
 GCGCGCGCGCGAATCTGCATACCGGAGACTTTCCAAACCCAAATGACGCATACAAGAATTTGATCAACCTCAAACCT
 GCAATTGAAGCAGGTATACAGGACGCGGGGTAGAGGTAGGTATCGTCGACACAGGCGAATCCGTGGCAGCATATCC

TTTCCGAACTGTATGGCAGAAAGAACACGGCTATAACGAAATTACAAAACTATACGGCGTATATGCGGAAGGAA
 GCGCTGAAGACGAGGCGGTAAAGACATTGAAGCTTCTTTTCGACGATGAGGCCGTTATAGAGACTGAAGCAAAGCCG
 ACGGATATCCGCCACGTAAAAGAAATCGGACACATCGATTGGTCTCCCATATTATTGGCGGGCGTTCCGTGGACGGC
 5 AGACTGCGAGCGGTATTGCGCCCGATGCGACGCTACACATAATGAATACGAATGATGAAACCAAGAACGAAATGATG
 GTTGCAGCCATCCGCAATGCAATGGGTCAAGCTGGGCGAACGTGGCGTGGCATCGTCAATAACAGTTTGGAAACAACA
 TCGAGGGCAGGCACTGCCGACCTTTTCCAAATAGCCAATTTCGAGGAGCAGTACCGCCAAGCGTTGCTCGACTATTCC
 GGCGGTGATAAAACAGACGAGGGTATCCGCCTGATGCAACAGAGCGATTACGGCAACCTGTCTTACCACATCCGTAAT
 AAAAAACATGCTTTTCATCTTTTCGACAGGCAATGACGCACAAGCTCAGCCCAACACATATGCCCTATTGCCATTTTAT
 10 GAAAAAGACGCTCAAAAAGGCATTATCACAGTCGACGGCGTAGACCGCAGTGGAGAAAAGTTCAAACGGGAAATGTAT
 TGAGAACCGGGTACAGAACCGCTTGAGTATGGCTCCAACCATTTGCGGAATTACTGCCATGTGGTGCTGTGCGCACCC
 TATGAAGCAAGCGTCCGTTTACCCGTACAAACCCGATTCAAATTGCGGAACATCCTTTTCCGCAACCCATCGTAACC
 GGCACGGCGGCTCTGCTGCTGCAGAAATACCCGTGGATGAGCAACGACAACCTGCGTACCACGTTGCTGACGACGGCT
 CAGGACATCGGTGCAGTCCGCGTGACAGCAAGTTTCGGCTGGGACTGCTGGATGCGGGTAAGGCCATGAACGGACCC
 GCGTCTTTCCGTTTCGGCGACTTACCGCCGATACGAAAGGTACATCCGATATTGCCCTACTCTTCCGTAACGACATT
 15 TCAGGCACGGCGGCGCTGATCAAAAAAGGCGGCAGCCAATGCAACTGCACGGCAACAACACCTATACGGGCAAAACC
 ATTTATCGAAGCGGTTCGCTGGTGTGTACGGCAACAACAAATCGGATATGCGCGTCGAAACCAAGGTGCGCTGATT
 TATAACGGGGCGGCATCCGGCGGCAGCCTGAACAGCAGCGCATTGCTCTATCTGGCAGATAACCGCAATCCGGCGCA
 AACGAAACCGTACACATCAAAGGCAGTCTGCAGCTGGACGGCAAGGTACGCTGTACACAGTTTGGGCAAACTGCTG
 AAAGTGGACGGTACGGCGATTATCGGCGGCAAGCTGTACATGTGCGCACGGCAAGGGGGCAGGCTATCTCAACAGT
 20 ACCGGACGACGTGTTCCTTCTTGAGTGCCGCCAAAATCGGGCAGGATTATTCTTTCTCACAAACATCGAAACCGAC
 GCGGCGCTGCTGGCTTCCCTCGACAGCGTCGAAAAACAGCGGGCAGTGAAGGCGACACGCTGTCTATTATGTCCGT
 CGCGCAATTCGGCACGGACTGCTTCGGCAGCGGCACATTCCGCGCCCGCGGTCTGAAACACGCCGTAGAACAGGGC
 GGCAGCAATCTGGAACACCTGATGGTCAACTGGATGCCCTCCGAATCATCCGCAACCCGAGACGGTGAACCTGCG
 GCAGCCGACCGCACAGATATGCCGGGCATCCGCCCTACGGCGCAACTTTCCGCGCAGCGGCAGCGGTACAGCATGCG
 25 AATGCCGCGGACGGTGTACGCATCTTCAACAGTCTCGCCGCTACCGTCTATGCCGACAGTACCGCGCCCATGCCGAT
 ATGCAGGGACGCCGCTGAAAGCGGTATCGGACGGGTGGAACCAACGGCACGGGTGCGCGCTCATCGCGCAAAACC
 CAACAGGACGGTGAACGTGGGAACAGGGCGGTGTGAAGGCAAAATGCGCGGCAGTACCCAAACCGTCGGCATTTGCC
 CGGAAAACCGCGCAAAATACGACAGCAGCGCCACACTGGGCATGGGACGACGACATGGAGCGAAAAACAGTGAAT
 GCAAAAACCGCACAGCATTAGTCTGTTTTCAGGCATACGGCACGATGCGGGCGATATCCGCTATCTCAAAGGCTGTTT
 30 TCCTACGGACGCTACAAAAACAGCATCAGCCGACGACCGGTGCGGACGAACATGCGGAAGGCAGCGTCAACGGCACG
 CTGATGCGAGCTGGGCGCACTGGCGGGTGTCAACGTTCCGTTTGGCGCAACGGGAGATTGACGGTGAAGGCGGTCTG
 CGCTACGACCTGCTCAAACAGGATGCATTCCGCCGAAAAAGGCAGTGTCTTGGGCTGGAGCGGCAACAGCCTCACTGAA
 GGCACGCTGGTGGGACTCGCGGGTCTGAAGCTGTGCAACCCCTTGAGCGATAAAGCCGTCCTGTTTGAACGGCGGGC
 35 GTGGAACCGCAGCTGAACGGACGCGACTACCGGTAACGGCGGCTTTACCGCGCGACTGCGAGCAACCGGCAAGACG
 GGGGACGCAATATGCCGCACACCCGCTGTTGTCGGCGCTGGGCGCGGATGTGCGAATTTCGGCAACCGCTGGAACGGC
 TTGGCACGTTACAGCTACGCCGTTTCAAACAGTACGGCAACACAGCGGACGAGTGGCGTAGGCTACCGGTTCTGA
 CTCGAG

1 MKHFPSKVL TAILATFCSG ALAATNDDDV KKAATVAIAA AYNNGQEING
 40 51 FKAGETIYDI DEDGTITKKD ATAADVEADD FKGLGLKKVV TNLTKTVNEN
 101 KQNVDAKVA AESEIEKLTT KLADTDAALA DTDALDATT NALNKLGENI
 151 TTFAETKTN IVKIDEKLEA VADTVDKHAE AFNDIADSLD ETNTKADEAV
 201 KTANEAKQTA EETKQNVDAK VKAAETAAGK AEAAAGTANT AADKAEAVAA
 251 KVTDIKADIA TNKDNIAKKA NSADVYTREE SDSKFVRIDG LNATTEKLDLT
 45 301 RLASAEKSIA DHDTRLNGLD KTVSDLRKET RQGLAEQAAL SGLFPQYNVG
 351 GSGGGTSAP DFNAGGTGIG SNSRATTAKS AAVSYAGIKN EMCKDRSMLC
 401 AGRDDVAVD RDAKINAPPP NLHTGDFPNP NDAYKNLINL KPAIEAGYTG
 451 RGVEVGIVDT GESVGSISFP ELYGRKEHGY NENYKNYTAY MRKEAPEDGG
 501 GKDIEASFDD EAVIETBAKP TDIRHVKEIG HIDLVSHIIG GRSVDGRPAG
 50 551 GIAPDATHI MNTNDETKNE MMVAIRNAW VKLGERGVRI VNNSFGTTSR
 601 AGTADLFQIA NSEEQYRQAL LDYSGGDKTD EGIRLMQSD YGNLSYHIRN
 651 KNMLFIFSTG NDAQAPNTY ALLPFYEKDA QKGIITVAGV DRSGEKFKRE
 701 MYGBPGTEPL EYGSNHCGIT AMWCLAPYE ASVRFRTRNP IQIAGTSFSA
 751 PIVTGTALL LQKYPWMSND NLRTLLTTA QDIGAVGVS KFGWGLLDAG
 55 801 KAMNGPASFP FGDFADTKG TSDIAYSFRN DISGTGGLIK KGGSQLQLHG
 851 NNTYTGTII EGGSLVLYGN NKSDMRVETK GALIYNGAAS GGSLSNDGIV
 901 YLADTDQSGA NETVHIKSL QLDGKGTLYT RLKLLKVDG TAIIGKLYM
 951 SARGKAGYL NSTGRRVPFL SAKIGQDYS FFTNIETDGG LLASLDSVEK
 1001 TAGSEGDTLS YYVRRGNAAR TASAAHSAP AGLKHAVEQG GSNLENLMVE
 60 1051 LDASESATP ETVETAAADR TDMPGIRPYG ATFRAAAVQ HANAADGVRI
 1101 FNSLAATVYA DSTAAHADMQ GRRLKAVSDG LDHNGTGRLV IAQTQDGGT
 1151 WEQGGVEGKM RGSTQTVGIA AKTGENTTAA ATLGMRSTW SENSANAKTD
 1201 SISLFAGIRH DAGDIGYKLG LPSYGRYKNS ISRSTGADEH AEGSVNGTLM
 1251 QLALGGMNV PFAATGDLTV EGGLEYDLLK QDAFAEKSA LGWSGNSLTE
 65 1301 GTLVGLAGLK LSQPLSDKAV LFATAGVERD LNGRDYTVTG GFTGATAATG
 1351 KTGARNMPHT RLVAGLGADV EFGNGWNGLA RYSYAGSKQY GNHSGRVGVG
 1401 YRF*

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention. For instance, the use of proteins from other strains is envisaged [e.g. see WO00/66741 for polymorphic sequences for ORF4, ORF40, ORF46, 225, 235, 287, 519, 726, 919 and 953].

5

EXPERIMENTAL DETAILS

Cloning strategy and oligonucleotide design

Genes coding for antigens of interest were amplified by PCR, using oligonucleotides designed on the basis of the genomic sequence of *N. meningitidis* B MC58. Genomic DNA from strain 2996 was always used as a template in PCR reactions, unless otherwise specified, and the amplified fragments were cloned in the expression vector pET21b+ (Novagen) to express the protein as C-terminal His-tagged product, or in pET-24b+(Novagen) to express the protein in 'untagged' form (e.g. ΔG 287K).

Where a protein was expressed without a fusion partner and with its own leader peptide (if present), amplification of the open reading frame (ATG to STOP codons) was performed.

Where a protein was expressed in 'untagged' form, the leader peptide was omitted by designing the 5'-end amplification primer downstream from the predicted leader sequence.

The melting temperature of the primers used in PCR depended on the number and type of hybridising nucleotides in the whole primer, and was determined using the formulae:

$$T_{m1} = 4 (G+C) + 2 (A+T) \quad (\text{tail excluded})$$

$$T_{m2} = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad (\text{whole primer})$$

The melting temperatures of the selected oligonucleotides were usually 65-70°C for the whole oligo and 50-60°C for the hybridising region alone.

Oligonucleotides were synthesised using a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2.0ml NH₄OH, and deprotected by 5 hours incubation at 56°C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were centrifuged and the pellets resuspended in water.

		Sequences	Restriction site
fu (961)-	Fwd	CGCGGATCC -GGAGGGGGTGGTGTCTG	BamHI

741(MC58)-His			
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAGGC	XhoI
fu (961)-983-His	Fwd	CGCGGATCC - GGCGGAGGCGGCACTT	BamHI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
fu (961)- Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu (961 c-L)-741(MC58)	Fwd	CGCGGATCC -GGAGGGGGTGGTGTCTG	BamHI
	Rev	CCCGCTCGAG-TTATTGCTTGGCGGCAAG	XhoI
fu (961c-L)-983	Fwd	CGCGGATCC - GGCGGAGGCGGCACTT	BamHI
	Rev	CCCGCTCGAG-TCAGAACCGGTAGCCTAC	XhoI
fu (961c-L)-Orf46.1	Fwd	CGCGGATCCGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCGCTCGAG-TTACGTATCATATTTACGTGC	XhoI
fu-(ΔG287)-919-His	Fwd	CGCGGATCCGGTGGTGGTGGT-CAAAGCAAGAGCATCCAAACC	BamHI
	Rev	CCCAAGCTT-TTCGGGCGGTATTCGGGCTTC	HindIII
fu-(ΔG287)-953-His	Fwd	CGCGGATCCGGTGGTGGTGGT-GCCACCTACAAAGTGGAC	BamHI
	Rev	GCCCAAGCTT-TTGTTGGCTGCCTCGAT	HindIII
fu-(ΔG287)-961-His	Fwd	CGCGGATCCGGTGGTGGTGGT-ACAAGCGACGACG	BamHI
	Rev	GCCCAAGCTT-CCACTCGTAATTGACGCC	HindIII
fu-(ΔG287)-Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACGTGC	HindIII
fu-(ΔG287-919)-Orf46.1-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	HindIII
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu-(ΔG287-Orf46.1)-919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT-CAAAGCAAGAGCATCCAAACC	HindIII
	Rev	CCCGCTCGAG-CGGGCGGTATTCGGGCTT	XhoI
fu ΔG287(394.98)-...	Fwd	CGCGGATCCGCTAGC-CCCGATGTTAAATCGGC	NheI
	Rev	CGGGGATCC-ATCCTGCTCTTTTTTGCCGG	BamHI
fu Orf1-(Orf46.1)-His	Fwd	CGCGGATCCGCTAGC-GGACACACTTATTTGCGCATC	NheI
	Rev	CGCGGATCC-CCAGCGGTAGCCTAATTTGAT	
fu (Orf1)-Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACGTGC	HindIII
fu (919)-Orf46.1-His	Fwd1	GCGGCGTCGACGGTGGCGGAGGCACTGGATCCTCAG	SalI
	Fwd2	GGAGGCACTGGATCCTCAGATTGGCAAACGATTC	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
Fu (orf46)-287-His	Fwd	CGGGGATCCGGGGGCGGCGGTGGCG	BamHI
	Rev	CCCAAGCTTATCCTGCTCTTTTTTGCCGGC	HindIII
Fu (orf46)-919-His	Fwd	CGCGGATCCGGTGGTGGTGGTCAAAGCAAGAGCATCCA AACC	BamHI
	Rev	CCCAAGCTTCGGGCGGTATTCGGGCTTC	HindIII

Fu (orf46-919)- 287-His	Fwd	CCCCAAGCTTGGGGGCGGCGGTGGCG	HindIII
	Rev	CCCGCTCGAGATCCTGCTCTTTTGGCCGGC	XhoI
Fu (orf46-287)- 919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTCAAAGCAAGAGCAT CCAAACC	HindIII
	Rev	CCCGCTCGAGCGGGCGGTATTCGGGCTT	XhoI
(ΔG741)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(ΔG741)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
(ΔG741)-983-His	Fwd	GCGGCCTCGAG- GGATCCGGCGGAGGCGGCACTTCTGCG	XhoI
	Rev	CCCGCTCGAG-GAACC GG TAGCCTACG	XhoI
(ΔG741)-orf46.1- His	Fwd1	GGAGGCACTGGATCCTCAGATTTGGCAAACGATTC	Sall
	Fwd2	GCGGCGTTCGACGGTGGCGGAGGCACTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
(ΔG983)- 741(MC58)-His	Fwd	GCGGCCTCGAG-GGATCCGGAGGGGGTGGTGTGCGCC	XhoI
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAG	XhoI
(ΔG983)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(ΔG983)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
(ΔG983)-Orf46.1- His	Fwd1	GGAGGCACTGGATCCTCAGATTTGGCAAACGATTC	Sall
	Fwd2	GCGGCGTTCGACGGTGGCGGAGGCACTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI

* This primer was used as a Reverse primer for all the C terminal fusions of 287 to the His-tag.

[§] Forward primers used in combination with the 287-His Reverse primer.

NB – All PCR reactions use strain 2996 unless otherwise specified (e.g. strain MC58)

In all constructs starting with an ATG not followed by a unique *NheI* site, the ATG codon is
 5 part of the *NdeI* site used for cloning. The constructs made using *NheI* as a cloning site at the
 5' end (e.g. all those containing 287 at the N-terminus) have two additional codons (GCT
 AGC) fused to the coding sequence of the antigen.

Preparation of chromosomal DNA templates

N.meningitidis strains 2996, MC58, 394.98, 1000 and BZ232 (and others) were grown to
 10 exponential phase in 100ml of GC medium, harvested by centrifugation, and resuspended in
 5ml buffer (20% w/v sucrose, 50mM Tris-HCl, 50mM EDTA, pH8). After 10 minutes
 incubation on ice, the bacteria were lysed by adding 10ml of lysis solution (50mM NaCl, 1%
 Na-Sarkosyl, 50μg/ml Proteinase K), and the suspension incubated at 37°C for 2 hours. Two

phenol extractions (equilibrated to pH 8) and one CHCl_3 /isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes of ethanol, and collected by centrifugation. The pellet was washed once with 70%(v/v) ethanol and redissolved in 4.0ml TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The
5 DNA concentration was measured by reading OD_{260} .

PCR Amplification

The standard PCR protocol was as follows: 200ng of genomic DNA from 2996, MC581000, or BZ232 strains or 10ng of plasmid DNA preparation of recombinant clones were used as template in the presence of 40 μM of each oligonucleotide primer, 400-800 μM dNTPs solution, 1x PCR buffer (including 1.5mM MgCl_2), 2.5 units *TaqI* DNA polymerase (using
10 Perkin-Elmer AmpliTaq, Boehringer Mannheim ExpandTM Long Template).

After a preliminary 3 minute incubation of the whole mix at 95°C, each sample underwent a two-step amplification: the first 5 cycles were performed using the hybridisation temperature that excluded the restriction enzyme tail of the primer (T_{m1}). This was followed by 30 cycles
15 according to the hybridisation temperature calculated for the whole length oligos (T_{m2}). Elongation times, performed at 68°C or 72°C, varied according to the length of the Orf to be amplified. In the case of Orf1 the elongation time, starting from 3 minutes, was increased by 15 seconds each cycle. The cycles were completed with a 10 minute extension step at 72°C.

The amplified DNA was either loaded directly on a 1% agarose gel. The DNA fragment
20 corresponding to the band of correct size was purified from the gel using the Qiagen Gel Extraction Kit, following the manufacturer's protocol.

Digestion of PCR fragments and of the cloning vectors

The purified DNA corresponding to the amplified fragment was digested with the appropriate restriction enzymes for cloning into pET-21b+, pET22b+ or pET-24b+. Digested
25 fragments were purified using the QIAquick PCR purification kit (following the manufacturer's instructions) and eluted with either H_2O or 10mM Tris, pH 8.5. Plasmid vectors were digested with the appropriate restriction enzymes, loaded onto a 1.0% agarose gel and the band corresponding to the digested vector purified using the Qiagen QIAquick Gel Extraction Kit.

Cloning

The fragments corresponding to each gene, previously digested and purified, were ligated into pET21b+, pET22b+ or pET-24b+. A molar ratio of 3:1 fragment/vector was used with T4 DNA ligase in the ligation buffer supplied by the manufacturer.

- 5 Recombinant plasmid was transformed into competent *E.coli* DH5 or HB101 by incubating the ligase reaction solution and bacteria for 40 minutes on ice, then at 37°C for 3 minutes. This was followed by the addition of 800µl LB broth and incubation at 37°C for 20 minutes. The cells were centrifuged at maximum speed in an Eppendorf microfuge, resuspended in approximately 200µl of the supernatant and plated onto LB ampicillin (100mg/ml) agar.
- 10 Screening for recombinant clones was performed by growing randomly selected colonies overnight at 37°C in 4.0ml of LB broth + 100µg/ml ampicillin. Cells were pelleted and plasmid DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions. Approximately 1µg of each individual miniprep was digested with the appropriate restriction enzymes and the digest loaded onto a 1-1.5% agarose gel
- 15 (depending on the expected insert size), in parallel with the molecular weight marker (1kb DNA Ladder, GIBCO). Positive clones were selected on the basis of the size of insert.

Expression

- After cloning each gene into the expression vector, recombinant plasmids were transformed into *E.coli* strains suitable for expression of the recombinant protein. 1µl of each construct
- 20 was used to transform *E.coli* BL21-DE3 as described above. Single recombinant colonies were inoculated into 2ml LB+Amp (100µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100µg/ml) in 100ml flasks, to give an OD₆₀₀ between 0.1 and 0.2. The flasks were incubated at 30°C or at 37°C in a gyratory water bath shaker until OD₆₀₀ indicated exponential growth suitable for induction of expression (0.4-0.8 OD). Protein
 - 25 expression was induced by addition of 1.0mM IPTG. After 3 hours incubation at 30°C or 37°C the OD₆₀₀ was measured and expression examined. 1.0ml of each sample was centrifuged in a microfuge, the pellet resuspended in PBS and analysed by SDS-PAGE and Coomassie Blue staining.

Purification of His-tagged proteins

- 30 Various forms of 287 were cloned from strains 2996 and MC58. They were constructed with a C-terminus His-tagged fusion and included a mature form (aa 18-427), constructs with

deletions ($\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$) and clones composed of either B or C domains. For each clone purified as a His-fusion, a single colony was streaked and grown overnight at 37°C on a LB/Amp (100 µg/ml) agar plate. An isolated colony from this plate was inoculated into 20ml of LB/Amp (100 µg/ml) liquid medium and grown overnight at 37°C with shaking.

5 The overnight culture was diluted 1:30 into 1.0 L LB/Amp (100 µg/ml) liquid medium and allowed to grow at the optimal temperature (30 or 37°C) until the OD₅₅₀ reached 0.6-0.8. Expression of recombinant protein was induced by addition of IPTG (final concentration 1.0mM) and the culture incubated for a further 3 hours. Bacteria were harvested by centrifugation at 8000g for 15 min at 4°C. The bacterial pellet was resuspended in 7.5 ml of

10 either (i) cold buffer A (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8.0) for soluble proteins or (ii) buffer B (10mM Tris-HCl, 100 mM phosphate buffer, pH 8.8 and, optionally, 8M urea) for insoluble proteins. Proteins purified in a soluble form included 287-His, $\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$ 287-His, $\Delta 4$ 287MC58-His, 287c-His and 287cMC58-His. Protein 287bMC58-His was insoluble and purified accordingly. Cells were disrupted by

15 sonication on ice four times for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13000xg for 30 min at 4°C. For insoluble proteins, pellets were resuspended in 2.0 ml buffer C (6 M guanidine hydrochloride, 100 mM phosphate buffer, 10 mM Tris- HCl, pH 7.5 and treated with 10 passes of a Dounce homogenizer. The homogenate was centrifuged at 13000g for 30 min and the supernatant retained. Supernatants for both soluble and insoluble

20 preparations were mixed with 150µl Ni²⁺-resin (previously equilibrated with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 min. The resin was Chelating Sepharose Fast Flow (Pharmacia), prepared according to the manufacturer's protocol. The batch-wise preparation was centrifuged at 700g for 5 min at 4°C and the supernatant discarded. The resin was washed twice (batch-wise) with 10ml

25 buffer A or B for 10 min, resuspended in 1.0 ml buffer A or B and loaded onto a disposable column. The resin continued to be washed with either (i) buffer A at 4°C or (ii) buffer B at room temperature, until the OD₂₈₀ of the flow-through reached 0.02-0.01. The resin was further washed with either (i) cold buffer C (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8.0) or (ii) buffer D (10mM Tris-HCl, 100mM phosphate buffer, pH 6.3 and, optionally, 8M urea) until OD₂₈₀ of the flow-through reached 0.02-0.01. The His-fusion

30 protein was eluted by addition of 700µl of either (i) cold elution buffer A (300 mM NaCl, 50mM phosphate buffer, 250 mM imidazole, pH 8.0) or (ii) elution buffer B (10 mM Tris-HCl, 100 mM phosphate buffer, pH 4.5 and, optionally, 8M urea) and fractions

collected until the OD₂₈₀ indicated all the recombinant protein was obtained. 20µl aliquots of each elution fraction were analysed by SDS-PAGE. Protein concentrations were estimated using the Bradford assay.

Renaturation of denatured His-fusion proteins.

- 5 Denaturation was required to solubilize 287bMC8, so a renaturation step was employed prior to immunisation. Glycerol was added to the denatured fractions obtained above to give a final concentration of 10% v/v. The proteins were diluted to 200 µg/ml using dialysis buffer I (10% v/v glycerol, 0.5M arginine, 50 mM phosphate buffer, 5.0 mM reduced glutathione, 0.5 mM oxidised glutathione, 2.0M urea, pH 8.8) and dialysed against the same buffer for
10 12-14 hours at 4°C. Further dialysis was performed with buffer II (10% v/v glycerol, 0.5M arginine, 50mM phosphate buffer, 5.0mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was estimated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times OD_{280}) - (0.76 \times OD_{260})$$

Immunization

- 15 Balb/C mice were immunized with antigens on days 0, 21 and 35 and sera analyzed at day 49.

Sera analysis – ELISA

- The acapsulated MenB M7 and the capsulated strains were plated on chocolate agar plates and incubated overnight at 37°C with 5% CO₂. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into Mueller-Hinton Broth (Difco)
20 containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.4-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and bacteria were washed twice with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 1 hour at 37°C and then overnight at 4°C with stirring. 100µl bacterial cells
25 were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200µl of saturation buffer (2.7% polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN₃ in PBS) were added to
30 each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 100µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells

were washed three times with PBT buffer. 100µl of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenildiamine and 10µl of H₂O₂) were added to each well and the plates were left at room temperature for 20 minutes. 100µl 12.5% H₂SO₄ was added to each well and OD₄₉₀ was followed. The ELISA titers were calculated arbitrarily as the dilution of sera which gave an OD₄₉₀ value of 0.4 above the level of preimmune sera. The ELISA was considered positive when the dilution of sera with OD₄₉₀ of 0.4 was higher than 1:400.

Sera analysis – FACS Scan bacteria binding assay

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C with 5% CO₂. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD₆₂₀. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA in PBS, 0.4% NaN₃) and centrifuged for 5 minutes at 4000rpm. Cells were resuspended in blocking buffer to reach OD₆₂₀ of 0.05. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:100, 1:200, 1:400) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000rpm, the supernatant aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)₂ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan (Laser Power 15mW) setting were: FL2 on; FSC-H threshold:92; FSC PMT Voltage: E 01; SSC PMT: 474; Amp. Gains 6.1; FL-2 PMT: 586; compensation values: 0.

Sera analysis – bactericidal assay

N. meningitidis strain 2996 was grown overnight at 37°C on chocolate agar plates (starting from a frozen stock) with 5% CO₂. Colonies were collected and used to inoculate 7ml Mueller-Hinton broth, containing 0.25% glucose to reach an OD₆₂₀ of 0.05-0.08. The culture was incubated for approximately 1.5 hours at 37 degrees with shaking until the OD₆₂₀ reached the value of 0.23-0.24. Bacteria were diluted in 50mM Phosphate buffer pH 7.2 containing 10mM MgCl₂, 10mM CaCl₂ and 0.5% (w/v) BSA (assay buffer) at the working dilution of 10⁵ CFU/ml. The total volume of the final reaction mixture was 50 µl with 25 µl

of serial two fold dilution of test serum, 12.5 µl of bacteria at the working dilution, 12.5 µl of baby rabbit complement (final concentration 25%).

Controls included bacteria incubated with complement serum, immune sera incubated with bacteria and with complement inactivated by heating at 56°C for 30'. Immediately after the addition of the baby rabbit complement, 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method (time 0). The 96-wells plate was incubated for 1 hour at 37°C with rotation. 7µl of each sample were plated on Mueller-Hinton agar plates as spots, whereas 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method (time 1). Agar plates were incubated for 18 hours at 37 degrees and the colonies corresponding to time 0 and time 1 were counted.

Sera analysis – western blots

Purified proteins (500ng/lane), outer membrane vesicles (5µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded onto a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, using transfer buffer (0.3% Tris base, 1.44% glycine, 20% (v/v) methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with mice sera diluted 1:200 in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labelled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

The OMVs were prepared as follows: *N. meningitidis* strain 2996 was grown overnight at 37 degrees with 5% CO₂ on 5 GC plates, harvested with a loop and resuspended in 10 ml of 20mM Tris-HCl pH 7.5, 2 mM EDTA. Heat inactivation was performed at 56°C for 45 minutes and the bacteria disrupted by sonication for 5 minutes on ice (50% duty cycle, 50% output, Branson sonifier 3 mm microtip). Unbroken cells were removed by centrifugation at 5000g for 10 minutes, the supernatant containing the total cell envelope fraction recovered and further centrifuged overnight at 50000g at the temperature of 4°C. The pellet containing the membranes was resuspended in 2% sarkosyl, 20mM Tris-HCl pH 7.5, 2 mM EDTA and incubated at room temperature for 20 minutes to solubilise the inner membranes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, the supernatant was further centrifuged at 50000g for 3 hours. The pellet, containing the outer membranes

was washed in PBS and resuspended in the same buffer. Protein concentration was measured by the D.C. Bio-Rad Protein assay (Modified Lowry method), using BSA as a standard.

Total cell extracts were prepared as follows: *N. meningitidis* strain 2996 was grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl.

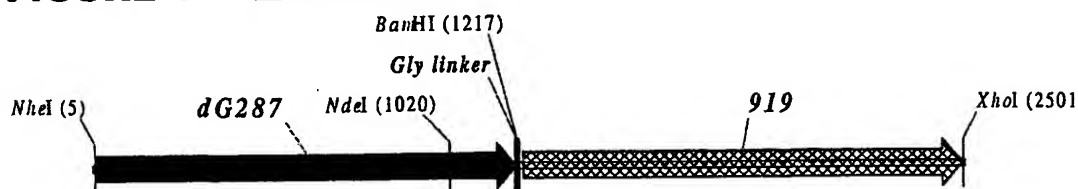
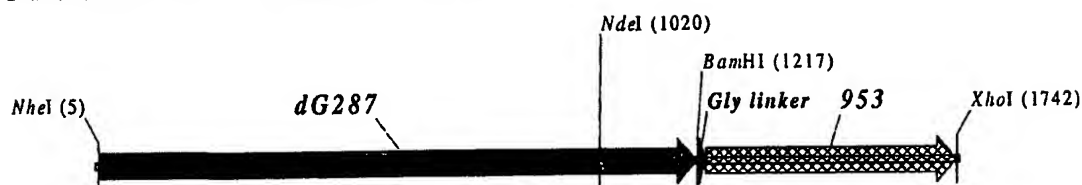
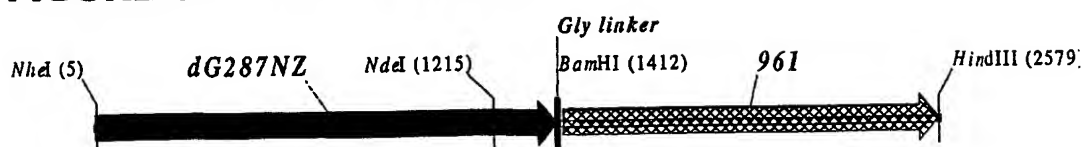
- 5 Heat inactivation was performed at 56°C for 30 minutes.

CLAIMS

1. A method for the simultaneous heterologous expression of two or more proteins of the invention, in which (a) two or more proteins of the invention are fused.
2. The method of claim 23, in which the two or more proteins are: (a) 919 and 287; (b) 953
5 and 287; (c) 287 and ORF46.1; (d) ORF1 and ORF46.1; (e) 919 and ORF46.1; (f)
ORF46.1, 287 and 919; (g) 919 and 519; and (h) ORF97 and 225.
3. The method of claim 24, in which 287 is at the C-terminal end of protein (a), (b) or (c).
4. The method of any preceding claim, in which the expression is in an *E.coli* host.
5. A protein expressed by the method of any preceding claim.
- 10 6. A hybrid protein of formula $\text{NH}_2\text{-A-B-COOH}$, wherein A and B are different Neisserial
proteins.
7. The protein of claim 6, wherein A and B are each selected from orf1, orf4, orf25, orf40,
orf46, orf83, 233, 287, 2921, 564, 687, 741, 907, 919, 953, 961 and 983.
8. The protein of claim 7, wherein A and B are each selected from ORF46.1, 287, 741, 919,
15 953, 961 and 983.
9. The protein of claim 8, wherein at least one of said ORF46.1, 287, 741, 919, 953, 961
and 983 is used in essentially full-length form
10. The protein of claim 8 or claim 9, wherein at least one of said ORF46.1, 287, 741, 919,
953, 961 and 983 has a deletion.
- 20 11. The protein of claim 10, wherein A and/or B has a poly-glycine deletion (' ΔG ').
12. The protein of claim 11, wherein A and/or B is $\Delta\text{G-287}$, ΔGTbp2 , ΔG741 , or ΔG983 .
13. The protein of claim 10, wherein A and/or B is a truncated protein.
14. The protein of claim 13, wherein A and/or B is $\Delta\text{1-287}$, $\Delta\text{2-287}$, $\Delta\text{3-287}$ or $\Delta\text{4-287}$.
15. The protein of claim 10, wherein a domain of A and/or B is deleted.

16. The protein of claim 15, wherein A and/or B is 287B, 287C, 287BC, ORF461-433, ORF46433-608, ORF46, or 961c.
17. The protein of claim 6, wherein A and B are: (a) 919 and 287; (b) 953 and 287; (c) 287 and ORF46.1; (d) ORF1 and ORF46.1; (e) 919 and ORF46.1; (f) ORF46.1 and 919; (g) 919 and 519; or (h) ORF97 and 225.
18. The protein of claim 17, wherein the protein is Δ G287-919, Δ G287-953, Δ G287-961, Δ G983-ORF46.1, Δ G983-741, Δ G983-961, Δ G983-961C, Δ G741-961, Δ G741-961C, Δ G741-983, Δ G741-ORF46.1, ORF46.1-741, ORF46.1-961, ORF46.1-961C, 961-ORF46.1, 961-741, 961-983, 961C-ORF46.1, 961C-741, 961C-983, 961CL-ORF46.1, 961CL-741, or 961CL-983.
19. The protein of claim 8, wherein A or B is 287.
20. The protein of claim 19, wherein B is 287
21. The protein of claim 19, wherein A is Δ G-287
22. The protein of claim 21, wherein B is ORF46, 919, 953 or 961.
23. The protein of any one of claims 19 to 22, wherein 287 is from strain 2996 or 394/98.
24. The protein of claim 8, wherein A is 961.
25. The protein of any one of claims 6 to 24, wherein A and B are from the same strain.
26. The protein of any one of claims 6 to 24, wherein A and B are joined directly
27. The protein of any one of claims 6 to 24, wherein A and B are joined via a linker peptide.
28. The protein of claim 27, wherein the linker peptide is a poly-glycine linker, with the proviso that B is not a Δ G protein.

1/5

FIGURE 1 — ΔG287—919**FIGURE 2 — ΔG287—953****FIGURE 3 — ΔG287—961****FIGURE 4 — ΔG287NZ—919****FIGURE 5 — ΔG287NZ—953****FIGURE 6 — ΔG287NZ—961**

BEST AVAILABLE COPY

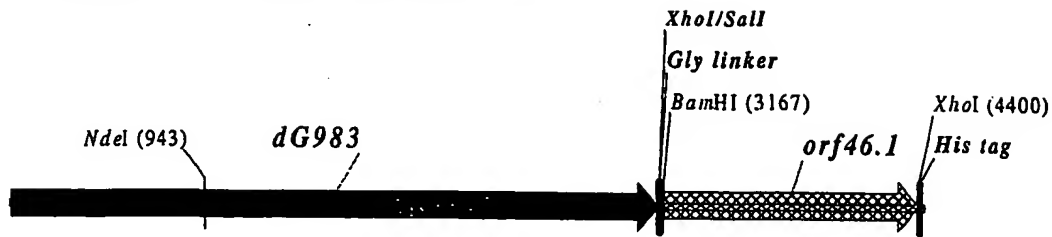
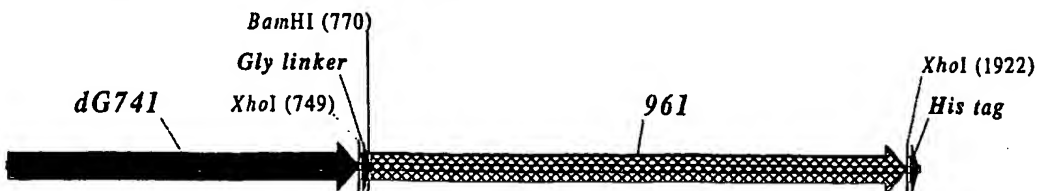
FIGURE 7 — ΔG983-ORF46.1**FIGURE 8 — ΔG983-741****FIGURE 9 — ΔG983-961****FIGURE 10 — ΔG983-961c****FIGURE 11 — ΔG741-961**

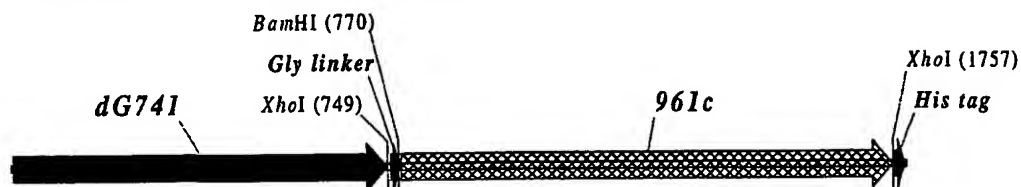
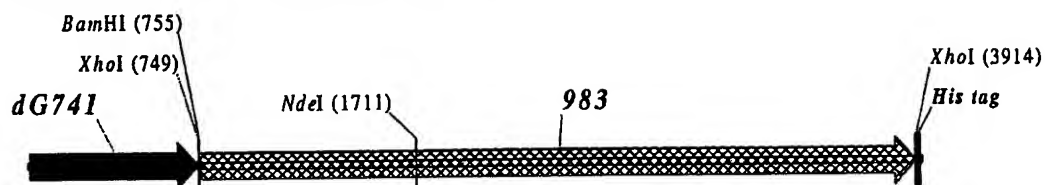
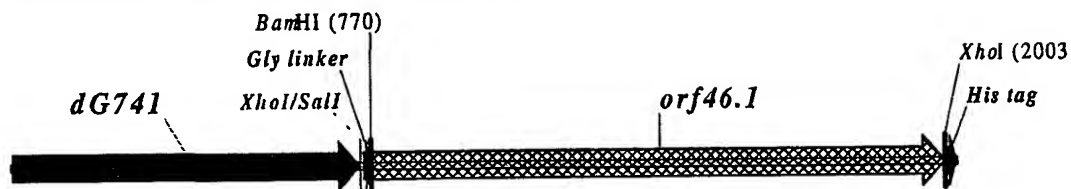
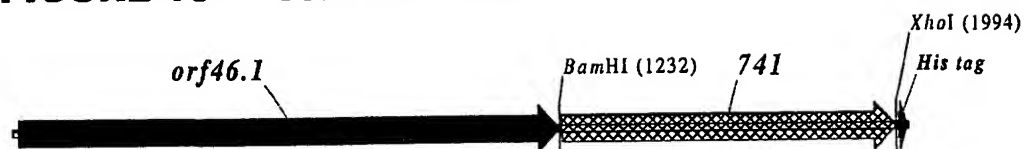
FIGURE 12 — ΔG741-961c**FIGURE 13 — ΔG741-983****FIGURE 14 — ΔG741-ORF46.1****FIGURE 15 — ORF46.1-741****FIGURE 16 — ORF46.1-961**

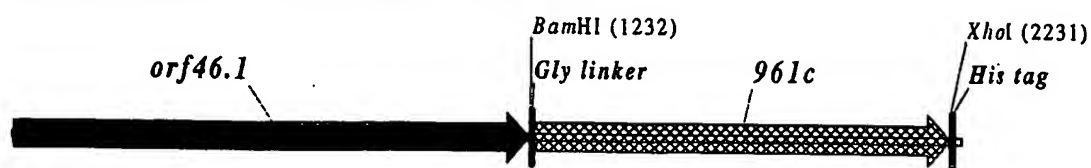
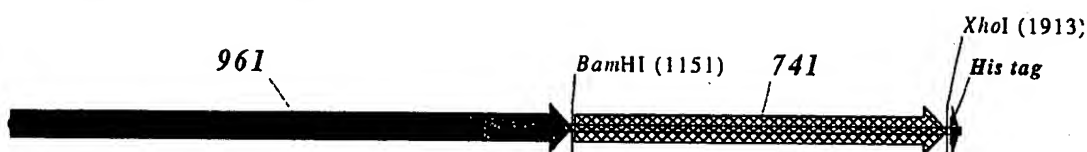
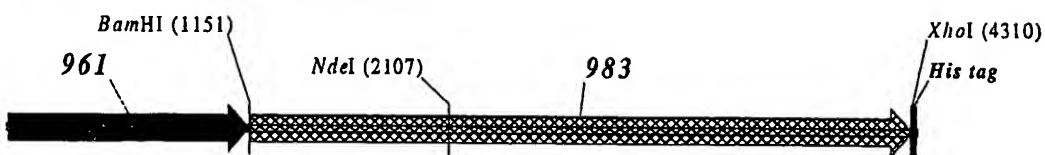
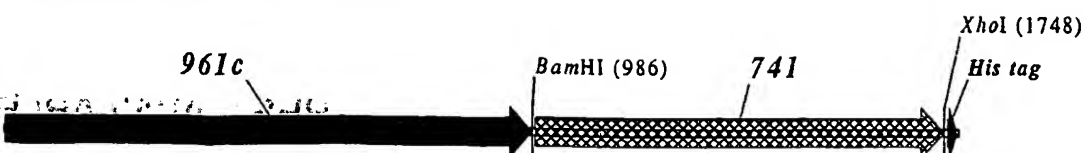
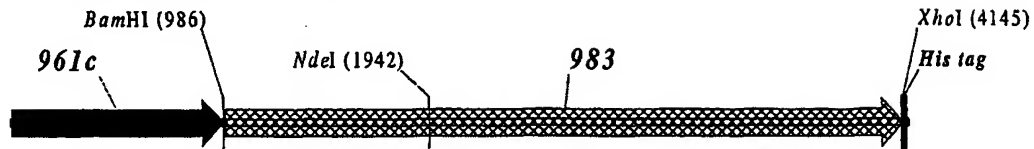
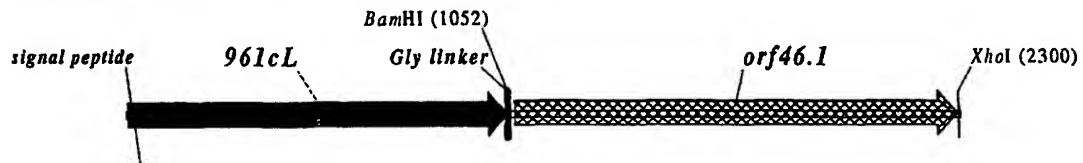
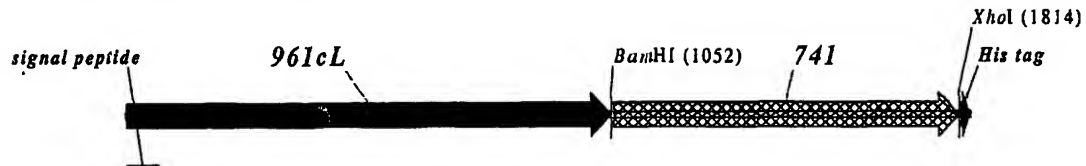
FIGURE 17 — ORF46.1—961c**FIGURE 18 — 961-ORF46.1****FIGURE 19 — 961-741****FIGURE 20 — 961-983****FIGURE 21 — 961c-ORF46.1****FIGURE 22 — 961c-741**

FIGURE 23 — 961c-983**FIGURE 24 — 961cL-ORF46.1****FIGURE 25 — 961cL-741****FIGURE 26 — 961cL-983**